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THE GENUS OPISTHORCHIS

BY

ARSHAG KILLIJIAN SEUERIAN

A. B. Ripon College, 1918

THESIS

Submitted in Partial Fulfillment of the Requirements for the

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I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY

SUPERVISION BY Arshag Killijian Seuerian

ENTITLED The Genus Opiathorchis

BE ACCEPTED AS FULFILLING THIS PART OF THE REQUIREMENTS FOR

THE DEGREE OF Master of Arts

Henry B. Ward

In Charge of Thesis

Henry B. Ward

Head of Department

Recommendation concurred in*

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on
Final Examination*

*Required for doctor's degree but not for master's

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UNIVERSITY OF ILLINOIS
THE GRADUATE SCHOOL

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I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY

SUPERVISION BY Alfred William Sessler

ENTITLED The Gamma Oligoethers

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THE DEGREE OF Master of Arts

Alfred W. Sessler
Chairman of Thesis
Alfred W. Sessler
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Recommendation contained in

Chairman
of
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INTRODUCTION

TABLE OF CONTENTS

I. Introduction

II. History

III. Synopsis of the species once named in the genus

Opisthorchis; and their division into three Genera:

Opisthorchis, Amphimerus and the genus Parapisthorchis

IV. Discussion

V. Description of Opisthorchis viverrini Var. Chiengmai

1. Material and Technique

2. History

3. General description of the form

4. The excretory system

5. The reproductive system

6. The alimentary system.

7. The body layers, glands and parenchymas

8. The nervous system

9. Discussion.

VI. Bibliography

VII. Explanation of Plates

INTRODUCTION

This investigation has been carried on under the direction of Dr. H. B. Ward with material furnished by him. The bottle containing the material was labeled *Opisthorchis viverrini* (?). The specimens were collected in 1915 by Dr. C. W. Mason from the liver of a prisoner who died in the Chiengmai jail, Siam. Hundreds of these forms were sent to Dr. Ward.

Leiper 1915 made a provisional diagnosis of this form and called it *Opisthorchis viverrini*.

The object of the present paper is two-fold: first to give a detailed description of this form; second, to bring all data available on the genus *Opisthorchis* in a single work for a ready reference in the study of the problem of species and varieties among the closely related species now existing.

Technique. - The specimens were somewhat poorly preserved. The material was stained in toto with Erlich's acid hematoxylin, borax carmine, and fuchsin. Cross, frontal and sagittal sections were made and stained with dilute Delafield's hematoxylin and with Erlich's acid hematoxylin counterstained with eosin. A great number of specimens were examined for marked variations. Twenty specimens were however selected at random for general measurements.

INTRODUCTION

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II. HISTORY

Loose (1899) made a few genera out of various species classified under the genus *Opisthorchis*. He placed these genera (*Holometra*, *Metorchis*, *Clonorchis* and *Opisthorchis*) under the subfamily *Opisthorchiinae*. The specific diagnosis of this new subfamily *Opisthorchiinae* Loose (1899:563) is as follows:

Fasciolidae of medium size with a slender elongated body, anterior end noticeably tapering. Suckers near one another and usually not well developed. Pharynx present. Intestinal crura long and simple, short and slender oesophagus. Excretory bladder Y-shaped with a long S-shaped branch winding between the testes and with short side branches. Genital pore commonly in front of the acetabulum. Copulation organs absent. Testes near the hinder end more or less obliquely placed. Ovary is in front of the testes. Laurer's canal present. Seminal receptacle very strongly developed. Uterus loops in front of the testes. Vitellaria moderately developed, lateral to the intestinal crura.

Found in the liver of mammals, birds and reptiles.

The above diagnosis and the three new genera belonging to the new subfamily, in its modified form has been accepted by all parasitologists.

Campula was originally proposed for distomes with a wavy intestine by Cobbold (1885). Stiles and Hassall did not find sufficient ground for the separation. "*Opisthorchis* was based upon the topography of the genital organs, *Campula* upon the crooked intestine. The two genera are, however, hardly to be separated at present, since *Campula* agrees in topography with *Opisthorchis*"

(Paras. 1:85). Later Cobbold himself admitted the identity of the two species (1885:39). Looss expresses the same opinion as Stiles and Hassall (1889)

Key to the genera of the subfamily Opisthorchiinae Looss(1899)

1. (2) Vitellaria lateral to intestinal crura - - - - - 3

2. (5) Vitellaria anterior to intestinal crura, lateral to oesophagus; uterine coils fill entire body posterior to acetabulum - - - - - HOLOMETRA

3. (2) Vitellaria not extending anteriorly beyond the acetabulum - - - - - 4

4. Vitellaria extending beyond the acetabulum -- - - - -METORCHIS

5. (6) Testes dendritic - - - - - 7

6. Testes not dendritic - - - - - OPISTHORCHIS

7. Testes dendritic - - - - - CLONORCHIS

The diagnosis of the genus Opisthorchis Blanchard and the modifications made by Looss are placed below for comparison.

Opisthorchis R. Blanchard (1895:217). - Medium and large forms. Oesophagus long or short, occasionally wanting. Intestinal branches not ramified. Genital orifice in front of acetabulum. No cirrus pouch. Vitelline glands generally do not extend behind the testes. Ovary situated posterior to uterine coils. Testes simple, globular and ramified.

Genus Opisthorchis Blanchard in partim.- Body transparent, often very strongly attenuated, posterior end bread; tapering toward the anterior end. Skin smooth with or without spines.

Testes more or less strongly lobed or ramified, lie obliquely one in front of the other. Ovary lobed or simple. Uterus loops lying in front of the ovary and not usually reaching out over the intestinal crura. Vitellaria does not extend forward beyond the ventral acetabulum.

The type of the genus is *Opisthorchis felineus*.

From the above diagnosis of the genus *Opisthorchis* I leave out species once reported as *Opisthorchis* which do not agree to the modified genus *Opisthorchis*. For example *D. albidus* Braun (1893), according to the later modification of *Opisthorchis* Blanchard, falls into the genus *Metorchis* Looss (1899). *O. exigua* Muhl. (1898) comes under *Holometra*. *O. sinensis* Cobbold (1815) under *Clonorchis*. In the genus *Metorchis* are *O. crassiuscula* Rud. 1809 var. (? = sp. nov. ?) Janus. *O. crassiuscula* Janus Kowal. (1898h), *O. crassiuscula* Looss (1899b:678), *O. xanthosoma* Crepl? Wag., var. (?) *campascua*.

The species belonging to the genus *Opisthorchis* at present are as follows:

- O. felinus* Riv. 1884
- O. tenuicollis* Rud. 1819
- O. viverrini* Poirier 1886
- O. longissimus* Linst. 1883
- O. simulans* Looss 1896
- O. geminus* Looss 1896
- O. pisciocola* Odhner 1902
- O. obsequens* Nicoll 1915
- O. entzi* w. Ratz 1900

- O. interruptus Braun 1901
- O. lancea Diesing 1850
- O. noverca Braun 1902
- O. pseudofelineus Ward 1895
- O. speciosus Stiles and Hassall 1896
- O. ovalis Barker 1911

- O. caninus Barker 1911

III. SYNOPSIS OF THE SPECIES ONCE NAMED IN THE GENUS
OPISTHORCHIS: AND THERE DIVISION INTO THREE GENERA: OPISTH-
ORCHIS, AMPHIMERUS, AND PARAPISTHORCHIS.

(Ward 1903 and Stephens 1916)

The type species designated for the genus *Opisthorchis* is *O. felineus* Rivolta (1884). Gurlt named the fluke *Distoma conus* in 1831, but Creplin had already used the name for another species in 1825. Von Siebold's *Distoma lanceolatum* 1836, was preoccupied by the genus known as *Dicrocoelium dendriticum*. *Distoma sibiricum* proposed by Winogradoff 1892 cannot be accepted by law of priority and in fact it is a variety of *O. felineus* Riv. Rivolta gave *D. felineus* in 1884 which holds. *D. tenuicolle*, Mühl. 1896.

Diagnosis - Body much flattened, yellowish in color and almost transparent in living specimens. The sides of the body are parallel save in the region anterior to the acetabulum which is conical. There is a shallow constriction at the level of the ventral sucker noticeable in fresh and contracted specimens. The posterior end is either pointed or rounded off. The length and breadth vary according to the contraction, being usually 8 to 11mm by 1.5 to 2 mm. The suckers are about one-fifth to one-sixth of the body length distant from each other, and about equal in size, 0.23 to 0.25 mm. The oesophagus is very short. The intestinal crura reach almost to the posterior border and are often filled with blood. The excretory pore is at the posterior extremity, and the excretory bladder forks in front of the anterior testis. Anterior testis four lobed, posterior five lobed, and occupy the posterior fourth of the body one placed obliquely in front of the other. Ovary median, transverse, simple or slightly lobed.

Smaller than the pear or retort-shaped receptaculum seminalis. Laurer's canal present. Uterus in the median field and entirely within the intestinal crura. The vitellaria occupy the fairly broad lateral areas in about the central third of the body, beginning behind the ventral sucker and terminating at about the level of the ovary. The acini are small and are arranged in groups of seven to eight, separated by interstices. The genital pore is immediately preacetabular. Eggs oval with distinct operculum at the pointed pole, 0.030 by 0.011 mm.

Habitat - Gall duct and gall bladder of cats, dogs, glutton, fox and man.

Distribution - Europe, (Germany, France, Holland, Scandinavia, Hungary and Russia) and Siberia.

Opisthorchis viverrini Poirier 1886

Body transparent, lanceolate, 6mm long and 2 mm broad. The dorsal surface a little concave the ventral surface flat. Suckers small and equal in size, 0.23 mm. Ventral sucker at the beginning of the anterior third. The distance between the two suckers 1.8 mm.

Genital orifice immediately above the ventral sucker. The length of the pharynx 0.1mm immediately posterior to the oral sucker, which has a wide opening. Oesophagus is relatively short 0.3 mm. The intestinal forks are simple and with a nearly constant diameter 0.1mm and terminate blindly at the extreme posterior end.

The ovary is situated in front of the testes and is multi-lobed. It extends transversely, having two well developed lobes on the left and two smaller ones on the right. The shell glands in this case present a peculiar aspect. They are not compact

but small unicellular isolated glands scattered in the parenchyma around the beginning of the uterus.

The racemose vitelline glands are situated laterally in the median region of the body and in front they disappear before reaching the level of the ventral sucker. The single transverse vitelline duct has the vitelline reservoir on the left of the shell gland. Laurer's canal very distinct at the base of which the pear shaped receptaculum seminalis is situated just outside of the shell gland. The uterus is very strongly coiled and situated in the region of the body between the ventral sucker and the ovary.

The eggs are small, ovoid, 0.026 mm long and 0.013 mm wide.

The male organs are composed of two small four lobed testes; which are situated at the posterior of the body. The vas deferens is a very narrow and long duct. It forms a coiled pouch at the right of the middle line of the body and extends to the genital cloaca.

The excretory apparatus is not clearly distinguished. The excretory bladder is quite voluminous and extends from the external pore at the posterior end to the ovary.

These were collected from the bile duct of *Felix viverrinus* which died in the cattle shed of a Museum in France. (Poirier 1886:8)

Opisthorchis geminus Looss (1896:50)

The body flattened, elongated, tapering anteriorly, increasing in size posteriorly. Total length 7 to 8 mm, in *Circus aeruginosis* 10 to 12.5 by 1 to 1.7 mm with a cephalad region 0.2 mm. The greatest width 1.3 mm. Oral sucker quite small 0.17

mm in diameter. The oral opening subterminal. Acetabulum smaller than the anterior sucker, situated at the second fourth of the body.

The integument very delicate, smooth and without any spicules or spinelysts.

Digestive system - The mouth is followed by the small pharynx measuring not more than 0.1 mm in diameter followed by the slender oesophagus 0.25mm long. The intestinal crura are not thicker than the oesophagus and extend almost to the posterior end bending slightly toward the median plane lying at the sides of the excretory bladder at the extreme posterior end.

The Y-shaped excretory system curved slightly forming an "S" between the testes. The short branches at the anterior end do not extend over the intestine.

Genital system - The genital orifice is marked by a small opening immediately anterior to the acetabulum. Copulatory organs not noticed. The two testes are not situated exactly at the middle line of the body. The anterior one is placed somewhat to the left, the posterior to the right. The anterior testis four, the posterior usually five lobed; their greatest diameter not exceeding 0.6 mm. Each gives a small vas efferens uniting at the middle of the body forming the vas deferens which is very large in diameter 0.1 mm, and very long. After many transverse loops which do not reach the intestinal branches laterally it opens into the genital sinus.

The ovary usually trilobed, situated in front of the testes, is generally smaller than the testes and nearly in the middle line of the body. Laurer's canal is prominently curved and filled with spermatozoa. In the interior of the germ duct cilia are

present. The vitellaria occupy the lateral portion of the body outside of the intestinal branches. The vitelline glands begin about the anterior third or middle of the length of the body and extend not further than the posterior end of the ovary. The transverse ducts consequently are posterior to the vitellaria. The course in fact is not perpendicular to the longitudinal axis of the body but extends somewhat posteriorly. The much coiled uterus does not extend over the intestinal branches. It opens into the genital sinus.

The eggs are oval in shape with a lid, 0.02 mm long and 0.01 mm wide. Perfectly developed embryos are seen in the metaterm region.

Habitat - In the biliary duct of *Milvus parasiticus*, *Anas boschas fera*, *Circus aeruginosus*.

Distribution - Egypt.

Opisthorchis simulans Looss (1896:52)

Three adult specimens and one young form found by Looss in the biliary duct of *Pernis apivorus* captured near Alexandria. The worms were located near the liver in the biliary passage.

The specific diagnosis - Body flat, elongated, anterior end less tapering, posterior end slightly tapering. The length is 7 to 23 mm, the greatest width across the middle of the body 1.5mm. The narrow anterior end 0.6 mm. The anterior sucker surrounding the oral opening is terminal, it is relatively large, strong and measures 0.5 mm in diameter, the acetabulum on the other hand is quite small and does not measure more than 0.2 mm in diameter, it is situated nearly at the level of the anterior third of the body.

The integument is smooth without spines or spinelets.

Digestive system - The pharynx well developed 0.3 mm in diameter nearly in contact with the bifurcation of the crura so that no oesophagus is present. The crura run to the posterior end of the body widening a little at the extremity.

The nervous system - The nervous system is similar to that of the other members of this genus.

The excretory system - The excretory system is Y-shaped. The unpaired bladder winds between the testes and has two short branches, into which opens a system of vessels at the sides of the ovary.

The genital system. - The genital orifice is at the anterior border of the acetabulum and opens into the genital sinus. The testes have a median position are spherical or elongated, 0.6 mm in diameter. The vasa efferentia start from the lateral anterior borders of the testes and join 0.8 mm posterior to the middle of the acetabulum. By this union they form in this species the single vas deferens 0.1 mm in maximum diameter which opens into the male orifice after having a winding course.

The ovary is quite small, lobed (follicular) and is situated in front of the testes at the left and adjoining the middle plane of the body. The receptaculum seminis is small and club shaped. Laurer's canal is quite short and a little swollen at the region which follows the receptaculum seminis. At the anterior portion of the oviduct as in other parts cilia are present. The vitellaria occupy the space at the sides of the body outside of the intestinal branches. They are very much ramified and extend to the anterior border of the anterior testis. The uterus occupies

the anterior portion of the female genital organs. The loops wind transversely and do not extend over the intestinal crura, it opens into the common genital sinus.

The eggs are larger than in *O. geminus* and measure 0.028 mm in length by 0.018 mm in width. The operculum is a little thinner. The shell is thin but less transparent on account of the brown color. Eggs in the region of the metraterm have not developed embryos.

Habitat - Liver of *Anas penelope*, *Fulix cristata*, *Circus aeruginosus* and *Pernis apivorus*.

Distribution - Egypt.

Opisthorchis obsequens Nicoll (1914:106)

The body is very much flattened with crenate edges. The anterior end is considerably attenuated but the posterior end is rounded. A small knob-like tip projects however from the anterior end. The adult specimens measure 2.6 to 5.0 mm. The maximum breadth which occurs in the post-acetabular region, varies from 0.8 to 1.2 mm. The average specimen measures 3.7 by 1. mm. There are no spines. The oral sucker is almost terminal and measures 0.16 by 0.20 mm on the average. Its transverse diameter varies from 0.15 to 0.24 mm. The ventral sucker measures 0.26 mm and is situated 1.24 mm from the anterior end, which is exactly one-third of the body length.

There is no prepharynx. The pharynx has a diameter of 0.12 mm and is slightly longer than broad. The oesophagus is about 0.19 mm in length with a range of 0.13 to 0.29 mm. The intestinal diverticula are sinuous and narrow with crenate walls, especially on their inner side. They extend to within 0.2 mm of the

posterior end of the body. Their ends are turned in towards the middle line.

The excretory vesicle has the usual sinuous configuration observed in the other species of the genus. The posterior curve may be either towards the right or to the left. The bifurcation occurs just behind the ovary and the limbs are short.

The genital aperture is median and immediately in front of the ventral sucker. The vesicula seminalis is short and highly convoluted. It extends beyond the ventral sucker for a distance about 0.3 mm. The testes are very much lobed, almost dendritic. There are usually five lobes on the anterior testis and four on the posterior; but this is not invariable. They are more or less tandem, while there is always a strong tendency to obliquity, the anterior testis being usually displaced towards the left side. Amphitypy occurs frequently. The posterior testicular space measures 0.34 mm which is about one-eleventh of the body length.

The ovary lies in front of the testes and is almost median. It is excessively lobed, there being usually three lobes directed backwards. It is much smaller than either of the testes. The receptaculum seminis is of median size, pear-shaped and lies as a rule on the right. The yolk glands extend from the level of the middle of the ventral sucker to the level of the anterior testis and usually not beyond. They lie entirely to the outer side of the intestinal diverticula and consist of eight groups of follicles on either side. The transverse yolk ducts meet at a very obtuse angle in front of the ovary. The uterus entirely fills the space between the ovary and the ventral sucker. It forms from the fourteen to sixteen transverse convolutions which occasionally touch, but do not overlap, the intestinal diverticula

The egg measures 0.025 to 0.030 by 0.014 to 0.017 mm the average being 0.028 by 0.0155 mm.

Habitat - Liver of *Hieracidea berigora* (brown Hawk) and
Hieracidea orientalis.

Distribution - North Queensland.

Opisthorchis entze Von Ratz, (1900: 532-534)

"Body flattened dorso-ventrally, lanceolate in shape and transparent; anterior portion whitish and posterior brownish yellow; anterior end tapering and constricted at the level of the acetabulum forming a neck region anterior to the acetabulum; posterior portion of the body broader and the end rounded; length 12 to 13.2 mm; breadth at the widest part 1 to 1.2 mm. Oral sucker 0.25 mm in diameter; acetabulum 2.38 mm posterior to the oral sucker and a little larger, measuring 0.31 mm in diameter. Pharynx oval or heart shaped and followed by a short oesophagus and forked intestinal tract, the crura ending in blind enlargements in the posterior end of the body. Cirrus and cirrus sac absent. Testes lie in the posterior half of the body, the one behind the other, 4 to 5 lobed. Vas efferens from each testes unite to form the vas deferens which passes forward to the genital pore situated just anterior to the acetabulum. Ovary in the middle of the body and slightly lobed, the retort shaped receptaculum seminis and tubular Laurer's canal just posterior to the ovary. Vitellaria lateral to the intestinal caeca and extend from near the acetabulum to the ovary. Acini of the vitelline glands somewhat indefinite being crowded together in spaces. The vitelline glands extend farther cephalad on the right side than of the left. The two vitelline ducts, one from each side, lead posteriorly to

the ovary where they unite. Uterine coils well developed and fill the body between the intestinal crura and the ovary and the acetabulum, but the coils do not extend over the intestinal crura. The shell gland is distinct and just anterior to the ovary. Eggs oval, more pointed at the anterior end where the operculum with an opercular rim is found. They measure 0.0242 mm by 0.0132 to 0.0154 mm".

Habitat - Blue Heron (*Ardea purpurea* L)

Distribution - Hungary.

Opisthorchis tenuicollis Rud. (1819:426)

Specific diagnosis - Body lanceolate, flat anterior end markedly tapering and thin, posterior end bluntly pointed; body 6.5 to 10.12 mm by 1.12 mm. Oral sucker 0.134 by 0.152 mm. Acetabulum 0.219 mm in diameter, situated between the first and second fourth of the body. Cuticula without spines. Ovary oval, smooth. Receptaculum seminis well developed. Laurer's canal and shell gland not mentioned. Testes in the posterior fourth of the body. Four to five lobed. Vitellaria in one region lateral to the intestinal caeca. Vitelline glands begin just posterior to the acetabulum and extend to the ovary. Uterus profusely developed. Eggs elliptical. 0.0228 by 0.014 mm.

Habitat - Reported from the domestic cat? dog? and man?

Gulo borealis, *Poca barbata*, *Halichorus grypus*,
Erignathus barbatus, *Dephinus phocaena*, *Phocaena phoc-*
aena.

Distribution. - Europe.

Opisthorchis piscicola Odhner (1902:152)

Body about 3 to 4 mm long. Greatest breadth about 0.5 mm

lies in the region of the middle of the body. From that the worm tapers strongly toward the anterior, much less strongly toward the posterior. The forward end is rounded. The first part of the posterior end behind the testes is abruptly pointed. Mouth opening circular 0.19 to 0.22 mm in diameter, ventral acetabulum transversely oval. \emptyset .25 by 0.3 mm. Situated in the end of the first body fifth. The body is armed with small broad spines. The pharynx is 0.08 mm in diameter. Testes strongly lobed lying in the median line. Ovary is more or less crenate. Genital glands immediately posterior to one another. The vitellaria extends from the posterior end of the ovary anteriorly to the middle third of the body. Ripe eggs 0.021 mm long and 0.011 mm broad.

Habitat - Gall bladder of Nile fish (*Gymnarchus niloticus*)

Distribution - White Nile. Omdurman.

Opisthorchis pseudofelineus Ward (1895:152-158)

Body elongated, transparent, flat; length 5 to 22 mm; breadth 1 to 2.5 mm. Anterior end conical; posterior end rounded. Oral sucker 0.24 to 0.541 mm. Ventral sucker 0.24 to 0.28 mm in diameter, situated about one-fourth of the total length of the body from the oral sucker. Pharynx 0.12 to 0.25 mm. long by 0.14 to 0.128 mm wide and next to the oral sucker, oesophagus short 0.04 to 0.38 mm in length; branches of the intestine extend nearly to the posterior end of the body. Two lobed testes in the posterior third or fourth of the body, the posterior lying obliquely caudad to the anterior. Anterior orbicular, oval or elliptical in shape; margin smooth, wavy or 2 to 5 lobed. Ovary elliptical, orbicular or oval in shape with margin smooth, wavy or lobed, situated a little in front of the testes. Receptaculum seminis pear, oval

or bag shaped, right or left of the ovary. Shell glands diffuse. Laurer's canal prominent. Uterus in coils from the ovary to the ventral sucker. Vitellaria lateral, occupying the middle third of the body and extending posteriad to the posterior testis. The transverse vitellogo duct passes from each region of the vitellaria ovarial and unites before or after reaching the ovary. The break of the vitellaria in front of the ovary varies in extent from 0.06 to 1.9 mm. Eggs long oval in shape, cap distinct with an opercular rim at the junction of the cap and the egg proper. Length 0.0286 to 0.0357 mm by 0.014 to 0.0162 in width. The excretory bladder is Y-shaped. The excretory canal sigmoid.. The genital pore at the anterior margin of the acetabulum. Skin provided by minute needle like spinelets on the anterior portion of the body as far caudad as the ovary. Spinelets deeply imbedded in the cuticula.

Habitat - Biliary ducts of the domestic cat and a pet Coyote.

Distribution - Nebraska and Iowa - U. S. A.

Opisthorchis interruptus Braun (1901:5)

The animals are flattened, of band like form, the sides of the body wavy, tapering anteriorly, posterior broader than the anterior. The extreme posterior end pointed. 7 to 8 mm in length; in the region of the acetabulum 0.5 mm in width; at the testes 0.7 mm. The oral sucker subterminal 0.25 to 0.26 mm broad, acetabulum 1.35mm in diameter, and 1.3 mm posterior to the oral sucker. A distinct prepharynx is wanting, the pharynx is 0.104 by 0.125 mm. The oesophagus has the same length as the pharynx. The two wide intestinal crura extend to the posterior end, one crus being longer than the other. The posterior testis lies in

front of the posterior body wall by a distance of 0.7 to 0.8 mm; diagonally to this the anterior testes; they are not of the same size and form, the anterior four lobed, the posterior is larger more nearly round and has less lobes. Between the testes runs the S-shaped excretory bladder. The receptaculum seminis is round or pear shaped and is smaller than the testes. Ovary median of the same size as the testes, oval in shape and lies just in front of the receptaculum seminis. The uterus coils transversely and is compact, the genital pore is situated immediately anterior to the acetabulum. A cirrus pouch is absent. The eggs are dark colored, 0.023 by 0.01 mm in size. The small vitelline follicles have a grouping distinctly as in the other species of the genus *Opisthorchis*; they begin anteriorly behind the acetabulum at a different level on each side, posteriorly they reach to the posterior testis. The distance of the anterior end of the vitellaria from the acetabulum is equal to the distance from the acetabulum to the oral sucker. It is characteristic that in the region of the receptaculum and as well in the ovarian region, an apparently long break in the vitellaria appears on both sides. This is approximately 0.6 mm long. The forms from the intestine of *Ardea viridirufa* appear to belong to the same species as they have the same length, the suckers and eggs are the same size and in every other respect correspond to *O. interruptus*, the vitellaria however could not be made out in those from *Alcedo virescens*.

Habitat - *Ardea viridirufa* and *Alcedo virescens*.

Distribution - Brazil.

Opisthorchis lancea Diesing (1850:579)

The body is lanceolate in form, neck and trunk regions present

The length of the body 5.5 to 12.5 mm. The greatest width 1. to 2.8 mm. The neck region is one third the total length of the entire body, is conical and elliptical in cross section. It carries on its forward end a subterminal oral sucker. The walls of the neck show a smooth wavy appearance, while that of the posterior end appears serrated. The oral sucker measures 0.33 to 0.36 mm by 0.56 to 0.66 mm. Acetabulum in anterior third of the body 0.51 to 1.2 mm in diameter and is more spherical than the oral sucker. A prepharynx is wanting. The oesophagus is short, intestinal crura reach to the posterior tapering end and run somewhat parallel. The excretory system is Y-shaped, the main branch winding in an S-shape between the testes to the excretory pore, which is terminal. The anterior testis is situated on the left and has four lobes, the posterior on the right with five lobes. The cirrus pouch is absent. The genital pore is situated median immediately anterior to the acetabulum. The receptaculum seminis is spindle formed, The ovary is biscuit shaped. The shell gland is indistinct. The eggs are oval 0.029 to 0.033 mm by 0.012 to 0.014 mm. The vitellaria lateral to the intestinal crura, extend from the posterior of the acetabulum to the level where the crura end. They are formed of small cross running acini, which combine in more or less distinct groups. On each side there are eight such groups of acini. The paired vitelline ducts begin usually between groups 4 and 5 of the acini, a space being here so that the vitellaria has two regions, and anterior ovarian and a posterior ovarian region. Amphitypy is evident in this species.

Habitat - Male *Delphinus tucuxi* (*Sleno tucuxi* Gray)

Orcella brevirostris

Distribution - Barra do Rio Negro, Brazil.

Opiethorchis ovalis, Barker (1911:518)

General shape of the body slender and oblanceolate, the anterior end tapering and bluntly conical, the posterior end broader and tapering slightly towards the end, There is a well defined narrower region or neck anterior to the acetabulum; the length of the body measures 6 to 14.32 mm; the breadth across the acetabulum 0.342 to 0.81 mm; across the ovary 0.45 to 1.373 mm. Small needle like, retrose spinelets abundant over the anterior two-thirds of the body, being most abundant at the anterior end as far caudad as the acetabulum and becoming sparser until the region of the ovary; in these specimens these spinelets were quite abundant. The oral sucker is subterminal 0.198 to 0.36 mm by 0.198 to 0.378 mm. The acetabulum in the anterior fifth, sixth, or seventh of the body, measuring 0.1331 to 0.216 mm by 0.131 to 0.225 mm. The genital pore immediately anterior and median to the acetabulum. The pharynx 0.188 to 0.15 mm by 0.108 to 0.162 mm. The oesophagus 0.09 to 0.63 mm in length. The intestinal crura are unequal in length and extend to the posterior end. The testes are in the posterior third of the body, oval ovate or orbicular, smooth or slightly lobed. A cirrus is absent. Ovary oval, ovate or orbicular, smooth or slightly crenate. The receptaculum prominent, two to four times the size of the ovary. Laurer's canal prominent. Shell gland diffuse and somewhat indistinct; uterine coils of median density and compactness. Vitellaria lateral to the intestinal caeca, divided into two distinct regions by a break near the ovary. Vitelline glands begin 0.36 to 1.89 mm posterior to the acetabulum and extend to

the posterior testis. Eggs short ovals in form, measuring 0.026 to 0.312 mm by 0.014 to 0.019 mm, with distinct lid but no opercular rim.

Habitat - Bile ducts of soft shell turtles (*Trionyx spinifer* Lesson and *Trionyx muticus* Lesseur) and *Chrysemys marginata*.

Distribution - Mississippi river in Minnesota and Iowa, U. S. A.

Opisthorchis noverca Braun (1902)

Syn:- *Distoma conjunctum* Lewis and Cunningham 1872

Distoma conjunctum McConnell 1876

Distoma conjunctum Cobbold 1859

Body lancet shaped, spinous, 9.5 to 12.7 mm long by 2.5 mm broad. Suckers close together, the oral being larger than the ventral. Pharynx spherical, intestinal crura extending far posteriorly. Genital pore immediately in front of the acetabulum. Testes round or only slightly lobed, situated at the limit of the posterior third of the body and nearly opposite. Ovary slightly lobed, in front of the bifurcation of the Y-shaped excretory reservoir. Uterus in loops through the central area from the ovary to the genital pore. Vitellaria lateral, beginning at the level of the acetabulum, extending to the middle of the posterior testis. A break of the vitellaria occurs in front of the ovary but it is indistinct. No cirrus sac. Eggs oval 0.034 by 0.021 mm.

Habitat - Gall ducts of man.

Distribution - India.

Opisthorchis caninus Barker (1911)

Syn:- *Distoma conjunctum* Lewis and Cunningham 1872

Opisthorchis noverca Braun 1903

Parppisthorchis caninus Stephens 1912

Opisthorchis caninus Barker 1911

Body slightly lanceolate, length varies from 2.75 to 5.75 mm in preserved specimens. Average length 3.6 to 5.2 mm. Body uniformly spinose. No spines on the pedicle. Oral sucker 0.28mm. Pharynx 0.224 by 0.184mm. Oesophagus 0.04 mm. The ventral sucker 0.176 mm in diameter. The acetabulum on the acetabular papilla between the first and second anterior fifth; smaller than the oral sucker. Pedicle about $\frac{1}{2}$ mm long. May be completely retracted. Genital pore on the apex of the pedicle in front of the acetabulum. Its exact position varies with the state of contraction of the parts. In certain cases it actually opens externally to the sucker and anterior to it. The opening is covered with scales. The vas deferens and uterus run alongside one another until they merge near the apex of the pedicle into a common sinus. The testes lie in the posterior third of the body, are orbicular or slightly lobed. One situated obliquely in front of the other. Ovary multilobular, the lobes 5 to 8, irregular in size and shape. It is located at the beginning of the posterior half. The seminal receptacle is globular, situated to the right of and dorsal to the posterior lobe of the ovary. Laurer's canal usually runs from the end of the receptacle with a single curve medially and backwards. The uterine coils form loosely packed transverse coils terminating slightly in front of the level of the first vitelline acinus. From here the uterus passes forward

into the pedicle to the left and ventral to the seminal vesicle. Seminal vesicle commences about the level of the first vitelline acini. The coils displace the uterus ventrally and to the left. In the pedicle the vesicle diminishes in extent and lies in its dorsal (anterior) side .

Habitat - Liver of Pariah dogs.

Distribution - India.

Opisthorchis speciosus Stiles & Hassall 1894

Syn:- *Distoma* (*Dicrocoelium*) *longissimum* var. *corvinus*
Stiles and Hassall 1894

Opisthorchis speciosus Stiles and Hassall 1896

Body elongated, its narrow and tapering anterior end covered with retrose spinelets, length 7.6 to 29 mm by 0.666 to 1.332 mm wide in the region of the ovary. Oral sucker subterminal, 0.291 to 0.366mm by 0.291 to 0.357 mm. Acetabulum in the anterior third or fourth of the body, 0.178 to 0.244 mm by 0.178 to 0.244 mm. The pharynx 0.15 to 0.205 mm by 0.131 to 0.188 mm. The oesophagus 0.15 to 0.30 mm long. Intestinal caeca equal or unequal, extend to the posterior end. Testes in posterior eight of the body, orbicular or oval, smooth or four lobed, Cirrus absent. Genital pore not prominent, at the anterior margin of the acetabulum. Ovary in posterior fifth of the body, oval, ovate or elliptical., smooth to three lobed. Receptaculum seminis smaller than or equal to the ovary. Shell gland diffuse or distinct. Laurer's canal prominent. Uterine coils very dense and close. Frequently extending laterally over the intestinal crura. Vitellaria divided into two distinct regions by a break in front of the ovary. Vitelline glands begin 0.203 to 0.263 mm posterior to the acet-

abulum and extend to the posterior testis. Eggs long ovals, with distinct lid and opercular rim, 0.025 to 0.033 by 0.015 to 0.018 mm

Habitat - Bile ducts of Crows (*Corvus americanus*, *Corvus ossifrage*) and the frog.

Distribution - Maryland and District of Columbia, U. S. A.

Barker, F. D. (1911) divided the genus *Opisthorchis* and proposed the generic name *Amphimerus* for those species which had two vitellaria regions caused by a break in the line of acini near the ovary, as postovarial and antovarial regions, while those forms which had only one region he retained in the old genus *Opisthorchia*.

Genus *Amphimerus* Barker (1911:536)

Generic diagnosis - Fasciolidae; body flattened, elongated, anterior end tapering. Skin frequently covered wholly or in part by small retrose spinelets. Excretory system Y-shape with sigmoid stem winding between the testes. Copulatory organs absent. Testes in posterior portion of the body, simple or lobate; the one obliquely posterior to the other. Ovary anterior to the testes, simple or lobate. Laurer's canal present; receptaculum seminis well developed. Uterine coils anterior to the ovary and may extend laterally over the intestinal caeca. Vitellaria well developed, lateral to the intestinal crura and divided into two distinct regions by a break opposite the ovary. Not extending anteriorly beyond the acetabulum but frequently extending posteriorly to or beyond the posterior testis.

Habitat - Parasitic in the gall ducts of mammals, birds and reptiles.

Type species - *Amphimerus ovalis* Barker (1911)

Stephens (1912) divided *Opisthorchis* once more retaining the

generic name for all the species except *Opisthorchis caninus* Barker which he placed under a new genus *Paropisthorchis*.

Genus *Paropisthorchis* Stephens (1912)

Structure as in *Opisthorchis*; except that the ventral sucker and genital pore occur on the apex of a process or pedicle projecting from the anterior portion of the body. This process is about 0.5 mm long and is retractile.

V. DISCUSSION

" It is a fact well acknowledged by persons who have worked with species of the genus *Opisthorchis*, that the forms in this genus represent one of the most difficult problems to the systematist", writes Looss (1899). " Several forms anatomically seem identical that should be classified as separate individuals on a biological basis" (1907). *Opisthorchis felineus* and *O. geminus* without knowledge of their host will be classified under the same species and in fact I am inclined to think *O. tenuicollis* is at most a variety of *O. felineus*. *O. felineus* is found in the liver of man and the cat in Europe; but *O. geminus* in Egypt in the liver of the non-migrating *Milvus parasiticus*, *Anas boschas fera*, *Circus aeruginosus*. *O. tenuicollis* from seals in Europe. Next to these three forms comes *O. viverrini* and *O. entzi* which differ from the above not in large details. Looss (1899) takes *O. geminus* as a separate species on biological grounds. The above mentioned birds are native to Egypt and never migrate and if they are the same as *O. felineus*, cats and man should be harboring these parasites, however this has not been reported nor has Looss been able to find any *Opisthorchis* in mammals contrary to his special search. *O. geminus* differs from the rest on the same ground as it differs from *O. felineus*. Looss again makes a comparison between *O. tenuicollis* and *O. geminus* from *Anas boschas fera*. *O. geminus* from *Anas* is 10. to 12.5 mm long and 1. to 1.7 mm broad; testes both lobed in *O. tenuicollis* and *O. geminus*. Ovary of *O. geminus* from two different kinds of hosts varies slightly from that of *Anas*, being round or slightly lobed, but

from *Circus* more distinctly lobed. *Vitellaria* same both in *O. geminus* and *O. tenuicollis*. The eggs of *O. tenuicollis* as given by Mühling 0.025 to 0.028 mm long and 0.013 mm wide, in *O. geminus* 0.027 by 0.013 mm in *Anas* and 0.023 by 0.013 mm in *Circus*. By such comparison Looss finds these forms identical yet thinks they cannot be classified as one species for the same reason as *O. felineus* cannot be the same as *O. geminus*, because of the specificity of the host. The same argument is brought about in regard to the nearly identical forms of *O. tenuicollis* and *O. felineus*. Braun in comparing the forms found by Mühling with *O. felineus* comes to the conclusion that they are different forms.

O. entzi has very identical characters with *O. tenuicollis* which in turn is closely related to *O. felineus* and *O. geminus*. It is taken as a separate species however by only specific differences of the suckers, in *O. entze* the acetabulum is larger than the oral sucker which in other forms is equal to it or the oral sucker is larger than the acetabulum. *O. viverrini* differs from the above mentioned form by the distinctly lobed ovary, by the moderately developed uterine coils and by the egg with an opercular rim. No figure accompanies Von Ratz's description of *O. entzi* which makes a comparison with *O. viverrini* less valuable.

The forms discussed above differ from each other by one or two characters in general. Two other forms which are more distinctly separated from the rest are *O. longissimus* and *O. simulans*. Both of these have ovary very much lobed (follicular). In the figure of Looss (1891:52) this character is not noticeable but his figure in a later description (1899:673) gives these characteristic lobes. The difference between *O. simulans* and *O.*

longissimus is quite small. *O. longissimus* tapers anteriorly but *O. simulans* only slightly. The reverse is the case posteriorly. The oral sucker in the later is much larger than the acetabulum, the reverse is the case with *O. longissimus*. The uterus coils are much closer in *O. longissimus* than in *O. simulans*. Testes in the former larger and somewhat lobed. Located more posteriorly than that in *O. simulans* which is more round, smaller and not not so much posterior. Some of these characters are much in variance as I have described in the case of *O. viverrini* var. *Chiengmai*. Yet the variations in the same species are within normal limit.

Opisthorchis obsequens Nicoll is distinguished by the smaller form, by its very much lobed testes which suggest the genus *Clonorchis*. The ovary is usually irregular in outline, ventral sucker is larger than the oral sucker; vitellaria more continued.

O. piscicola can be readily distinguished from the rest of the species by its minute size, with its relatively larger acetabulum. In this respect being much like *O. obsequens* but differs from it by its broad spines, lobed testes and its piscine host.

Another form described by Galli-Valerio (1898:145) and named as *O. pianae* is questioned by Kowalewski (1898: 751). Certain characters were overlooked which will make these forms identical with *E. conoideum*.

Galli-Valerio (1898:933) though admitting that this form may not belong to the genus *Opisthorchis* maintains that it is not identical with *E. conoideum*. From the description he regards it as *Opisthorchis* as it agrees with *O. Blanchard*.

From his figure it is certain that it does not belong to the genus *Opisthorchis* as the vitellaria extend to the posterior end of the body in an H-shape. However I cannot pass judgment regarding this form being an *Amphimerus* from his meager description. As Kowalewski has made a careful study of these forms it is safe not to include *O. pianae* in even *Amphimerus*.

Kowalewski (1898:52) described a form *O. simulans* var. *poturzyensis* from the liver of *Anas boschas dom.* He makes a comparison of this with *O. simulans* Looss and *O. longissimus*. His comparison is based upon the description of Looss (1896:52). Looss (1899:674) describes another form of *O. simulans* with slight variation from *Anas penelope* and *Felix cristata*. This form slightly larger than that from *Pernis apivorus* corresponds to Kowalewski's drawing (Fig. 23) and description.

Barker's division of *Opisthorchis* into two genera is questioned by some parasitologists. Leiper(have not been able myself to consult Leiper's article on this subject) has objected to such division on the ground that extent of the vitellaria posteriad - is a matter of chance and not a generic characteristic. I have examined more than fifty specimens of *O. viverrini* and I find the variation in the extent of the vitellaria small. They never extend behind the anterior testis, usually the vitellaria end at the level of the ovary. I have never noticed any break in the line in that region. The number of acini, their anterior or posterior extent, and the number of acini behind the transverse vitelline duct, does vary somewhat but always within a normal limit.

Mühling (1896:261) examined sixty specimens of *O. felineus* and from the description I find the variation in *O. felineus* the

same as in *O. viverrini*. *O. felineus* is the type species of the genus *Opisthorchis*. I find from the descriptions the same thing true for the vitellaria in all species of this genus as modified by Barker. The other species of *Opisthorchis* are classified under the genus *Amphimerus* Barker. This new genus is based entirely upon the division of the vitellaria into two regions, an antovarial and a postovarial with a distinct break in the line of acini at the ovary.

Weski (1900) examined 400 specimens of *O. lancea* Dies. for the extent of the variation of the vitellaria. In every case the vitellaria did extend caudad to the posterior testis which never was the case with *O. felineus* or the like. There was some irregularity in the line of acini opposite the ovary. In twenty-two cases only there was no break in the line of acini opposite the ovary on the right and only four on the left, but the acini were distinct. In 119 cases the groups coalesced on the right and in 35 on the left.

Ward (1894) describes the vitellaria in *Opisthorchis pseudo-felineus* as follows, "Vitellaria extend to the middle of the space between the two or even as far as the anterior edge of the posterior testis. One break in the line of acini may always be recognized as most prominent; it is located just opposite the ovary on each side, and is in length more or less equal to the diameter of the ovary. This is the condition shown on the left side of the worm figured. This space divides the vitellaria into two portions which may be distinguished as antovarial and postovarial. While it is in some cases possible to distinguish in the antovarial portion groups of acini they seem to be usually rather indistinct

or at least very unequal in size, as if adjacent groups had become confluent by the growth of interlying acini. The posterior portion, however is usually distinctly divided into two or three groups of acini, though even these may be obliterated. In two or three cases a small group of acini was found, on one side only in this intermediate space opposite the ovary, and was clearly separated from both antovarial and postovarial portions by a small space. This was the case in the right vitelline gland of the worm figured, corresponding to the two portions of glands, one finds on each side two ducts which, extending obliquely toward the ovary from a short distance before and behind it, form a "Y" or "V" according as they meet before or are not united after reaching the ovary. These ducts on either side of the ovary form one of the most characteristic appearances of the specimen".

Stiles (1904:32) describes these glands, " the antovarial portion four acini, postovarial three, each portion provided with its own oviduct, the antovarial portion extends from the ovary cephalad to about the boundary between the anterior and equatorial thirds of the body; the posterior portion extends caudad from the ovary to about the anterior plane of the posterior testis."

Barker (1907:100) makes a detail description of the variation of the vitellaria in *O. pseudofelineus* from more than a hundred specimens.

"The vitellaria never extend to the acetabulum, but their position posterior to posterior margin of the acetabulum varies on the right side from 0.3mm to 4.5 mm, the mode being 1.1 mm with frequency of 23 per cent. On the left side the position

varies from 0.3 mm to 3.7 mm the mode being 0.7 mm with a frequency of 18 per cent*****

***** The extent of the vitellaria caudad varies from the anterior margin of the anterior testis to a place 0.07 mm to 0.30 mm posterior to posterior testis, with a frequency of 28 per cent for the right side. On the left side the extremes were found to be a place 0.16 mm anterior to the posterior testis the mode being the anterior margin of the posterior testis, with a frequency of 22 per cent."

From the above quotations we find that the variation in the extent of the vitellaria in *O. pseudofelineus* is great but the break in the line of acini at the every quite constant. With all the variations *O. pseudofelineus* represents a homogeneous type which stands separately. Forms belonging to this type are *O. interruptus*, *O. lancea*, *O. noverca* and *O. speciosus* and *O. ovalis*, all having vitellaria divided into regions and a distinct break taking place at the ovarial region. In *O. noverca* the same is true except that the break in the line of acini at the ovary is less distinct. In this later form the vitellaria extends from lateral areas behind the acetabulum to the posterior testis.

Barker(1911) mentions that *Amphimerus noverca* is the only form which does not present a break in the line of acini in front of the ovary. But rightly he questions the accuracy of McConnell's drawing. It seems that Barker has not noticed the figure given by Leuckart. In this there is a distinct break in the line of acini in front of the ovary on one side.

The topographic details of the two groups are identical, they are parasitic in the same animals in the same organs. A study of

vitellaria among distomes in general shows forms in which the vitellaria varies from a knob like gland on each side to forms in which the vitellaria surrounds the whole body under the integument except a median dorso-ventral space. A typical type seems to be H-formed as is seen in *O. lancea*. It seems variation in the extent of the vitellaria comes by the degeneration of the glands as one of the possible causes. I have noticed in few specimens of *O. viverrini*, *Clonorchis endemicus* and *Clonorchis sinensis* that one or more acini are missing but the longitudinal duct is standing full of shell material. In other forms there were one or more acini below the transverse duct on one side and in others on the other side. The general position of the transverse duct was constant, though the ducts coming from each group of acini vary considerably. In *O. pseudofelineus* the vitellaria has four possible combinations both sides "V" shaped or "Y" shaped, right "V", left "Y" shaped or the reverse. The position of this transverse duct being constant, I find it is in the same place in both groups. Having all these variations and objections for the formation of a new genus *Amphimerus*, I find more argument in favor of such a division than against it. The genus *Meterorchis* is based chiefly on the extent of the vitellaria anterior to the acetabulum, the genus *Clonorchis* entirely on the dendritic testes. It is true that vitellaria extent posteriorly varies more than anteriorly but even so there is great constancy in this posterior extension. Among forms which form a homogeneous group *O. obaequens* Nicoll represents as much an intermediate form to the genus *Clonorchis* as the variation in the extent of the vitellaria does in *Opisthorchis*. Thus the forms *O. pseudofelineus*, *O. interruptus*, *O. lancea*, *O.*

noverca, *O. ovalis*, and *O. speciosus* became *Amphimerus pseudo-felineus*, *Amphimerus interruptus*, etc.,.

Amphimerus pseudofelineus, *A. ovalis* and *A. speciosus* are the three American species very much alike. Barker (1911:520) makes a detailed comparison based chiefly upon measurements some of them to tenths of a micron. Some of these measurements are confusing since a great range of variation is represented. The oral sucker in *A. ovalis* varies from 0.198 to 0.36 in length and in width from 0.198 to 0.378 mm, egg from 0.024 to 0.312 mm long by 0.014 to 0.019 mm wide. The mode of preservation might be one of the causes of variation in measurements. I find the egg one of the most constant characters in *O. viverrini*, *O. felineus* and *O. pseudo-felineus*, there are some abnormal eggs which must be rejected in measuring. The topography of these three American forms seem very much alike. Especially *A. ovalis* which is described as a new species chiefly because of the specificity of its host and some minor differences. Recently I have had for study a single specimen collected from *Chrysemys marginata* Agassiz taken near Fairport Iowa, which although poorly preserved, corresponds to the description of Barker except that the uterine coils are more compact, as in *A. speciosus*. Some of these differences according to Barker go more in variance among the individuals of the same species than between the species themselves. *A. ovalis* differs from the rest in its smaller size, the shape of the body being more slender, the oral sucker smaller, oesophagus larger, uterine coils more dense. *Amphimerus speciosus* has more characters in common with *A. pseudofelineus* than with *A. ovalis*; it differs however by the small size of the oral sucker,

the position of the ovary (at the posterior fifth of the body) and its large size; by the more densely coiled uterus and by its avian host. *A. ovalis* in general is smaller than *A. pseudofelineus* and *A. speciosus* is more slender has smaller oral sucker, larger oesophagus, position of the ovary more anterior (posterior half of the body), less dense uterus coils and smaller egg, constant oval shape of the ovary and testes and the reptilian host.

Amphimerus interruptus differs from the three American forms by its small size, 7 to 8 mm, its lobed testes, smaller and elong-egg, notably the difference in size of the oral and ventral suckers the former being much larger.

A. noverca once so much confused is easily distinguished from the above closely related forms by its general form of body which is smaller and broader, its very poorly developed uterine coils, its broad spinelets and the vitelline glands which are indistinct.

McConnell (1876:342) and (1878:476) found distomes in the biliary ducts of a Mohammedan, twenty eight years old who died in the medical college of Calcutta; and again in 1878 from another Mohammedan twenty four years old. McConnell diagnosed these forms as *D. conjunctum* Cobbold (1858). Lewis and Cunningham (1872:168) found in Pariah dogs in Calcutta, forms which they diagnosed as *Distomum conjunctum*. Stiles and Hassall (1894:430) showed that McConnell's form differed from that of Lewis and Cunningham. Looss (1899:565) placed Cobbold's *Distomum conjunctum* from the American red fox, *Canis vulpis* in the genus *Metorchis*. Braun (1903: 164) gave to McConnell's form the name *O. noverca*. Barker (1911:532) placed that of Lewis and Cunningham in the

genus *Opisthorchis* and named it *O. caninus*. Stephens (1912) placed *O. caninus* in a separate genus; *Paropisthorchis*. This new genus is based upon the 0.5 mm pedicle upon which is situated the acetabulum and the genital pore. In all forms of the genus *Opisthorchis*, the acetabulum is a cup shaped sessile adhesive organ sunken in the body parenchyma. In *Paropisthorchis* however the acetabulum and the genital pore are on a pedicle which is retractile. Such a character is a general one. In all other respect the genus *Paropisthorchis* is like that of *Opisthorchis*.

From the above discussion one thing is clear; that is the need of experimental work to clear the specificity of many of these forms. I am inclined to think such a work will bring the identity of *O. geminus*, *O. felineus*, *O. tenuicollis* and *O. entzi* under one species. *A. ovalis* and *A. speciosus* in another. Such an experiment can be done as suggested by Looss (1899) by feeding experimental animals. Another thing that will do much in proving or disproving the identity of these forms is careful search among hosts in question and a study of the life cycle in relation to the mode of transmission.

Key to the genera of the subfamily *Opisthorchiinae*

Looss 1899.

(See page 2 for diagnosis of the subfamily)

1. (2) Vitellaria lateral to intestinal caeca - - - - 3
2. (5) Vitellaria anterior to intestinal caeca, lateral to oesophagus uterine coils fill entire body posterior to acetabulum - - - - - HOLOMETRA
3. (2) Vitellaria not extending anteriorly beyond the acetabulum - - - - - 4

4. Vitellaria extending anteriorly beyond the acetabulum

METORCHIS

5. (6) Vitellaria not divided into two distinct regions
by a break in the line of acini near the ovary - 7

6. (5) Vitellaria divided into two distinct regions by a
break in the line of acini near the ovary -

b

AMPHIMERUS

7. (8) Testes not dendritic - - - - - 9

8. (7) Testes dendritic - - - - -

CLONORCHIS

9. (10) Acetabulum and genital pore on a pedicle

PAROPISTHORCHIS

10. (9) Acetabulum and genital pore not on a pedicle-

OPISTHORCHIS

Key to the species of the genus Amphimerus

Barker 1911

1. (2) Testes generally ~~double~~ - - - - - 3

2. (1) Testes generally simple, oval - - A. ovalis

3. (4) Coils of the uterus moderately developed - 5

4. (3) Coils of the uterus well developed A. speciosus

5. (6) Number of acini in postovarial portion of vitellaria
commonly four - - - - - m - 9

6. (5) Number of acini in postovarial portion of vitellaria
never more than three - - - - - 7

7. (8) A break in the line of acini moderate A. pseudofelineus

8. (7) Break in the line of acini indefinite
A. noverca

9. (10) Postovarial acini very marked - A. interruptus

10. (9) Break in the line of acini moderate A. landea

Key to the species of the genus Opisthorchis

- 1. (2) Body spineless - - - - - 3
- 2. (1) Body covered with spines - - - - -O. piscicola
- 3. (4) Suckers unequal, uterus well developed - 5
- 4. (3) Suckers equal uterus poorly developed
 - O. viverrini
- 5. (6) Ovary not multilobed - - - - - 9
- 6. (5) Ovary multilobed - - - - - 7
- 7. Oral sucker larger than acetabulum O. simulans
- 8. Oral sucker smaller than acetabulum
 - O. longissimus
- 9. (5)(12) Oral sucker smaller than the acetabulum- -10
- 10.(11) Found in the liver of Mammals - O. tenuicollis
- 11. (10) Found in the liver of birds - O. entz
- 12. (5)(9) Oral sucker larger than the acetabulum - 13
- 13. (14) Found in the liver of Mammals - -
 - O. felineus
- 14 (13) Found in the liver of Birds - O. geminus.

V. DESCRIPTION OF OPISTHORCHIS VIVERRINI

POIRIER 1886 VAR. CHIENGMAI

The general diagnosis - This parasite is leaf shaped, ob-lanceolate flattened dorso-ventrally. The preserved specimens are almost transparent, uterus region somewhat dark and always full of the numerous yellowish tinged eggs; the body has many ridges mostly passing transversely on account of the contraction. The integument is roughened by rubbing. The anterior third of the body starting at the acetabulum gradually tapers and is almost conical at the anterior end. At the level of the acetabulum on both sides of the body there is a slight shallow constriction which distinguishes a neck region. Posterior to the acetabulum the lateral borders run fairly parallel until to the posterior testis, from there it gradually decreases in width till at the extreme posterior end it tapers bluntly. The length and breadth vary according to the contraction and normal individual variation. The length varies from 2.7 to 7.7 mm. Two specimens are 6 mm in length, two 5, three 5.4 mm; four 4.6 mm. The average length of twenty specimens is 5.3 mm.

Width and thickness varies from 1 to 1.3 and 0.23 to 0.44 mm respectively. The suckers are about $\frac{2}{7}$ of the length of the body distant from each other. The variation is largely due to the expansion of the cephalic region at time of fixation. The suckers are about equal size 19 by 22.4 mm. The length of the oral sucker varies considerably from 0.125 to 0.23 mm. The width from 0.17 to 0.26 mm and 0.19 to 0.27 mm respectively. The acetabulum measures 22.5 by 23 mm. The oesophagus is much longer

than the pharynx, the former 0.31 mm on the average varying from 0.24 to 0.42 mm, the latter 0.14 by 0.13mm. The intestinal crura reach almost to the posterior border and are often filled with blood. The excretory pore is at the posterior extremity and the excretory bladder curves between the testes in an S-shape. The testes are located at the posterior, approximately $\frac{2}{7}$ of the body length from that end. They lie obliquely one behind the other; the anterior one has four lobes, the posterior one five. The ovary is multilobed with smooth margin; it occupies the anterior part of the posterior $\frac{1}{4}$ of the body (average) slightly toward the right (0.08) from the median. Behind the ovary lies the pear-shaped or retort-shaped seminal receptacle. Laurer's canal present. The uterus coils occupy a median position in the middle third of the body. The vitellaria occupy the fairly broad lateral areas, in about the middle five, six and seven and a half tenth's of the body. The number of acini varies from 7 to 9 usually 8 on each side. It never passes posteriorly beyond the anterior margin of the first testis nor reaches to the acetabulum. Cirrus pouch absent. The eggs are oblique oval with a round operculum at the sharper end 0.026 by 0.0156 mm. There is a distinct narrowing towards the anterior end, a distinct lid followed by a sharply projecting brim as in *Clonorchis sinensis*.

Habitat - Liver.

Host - Indian civet cat, first time recorded from man in
Siam 1915

I included the above short description in order to give a general idea of the form; but it seems to me that minor details

need to be mentioned though some of them are in considerable variance, for easy diagnosis by later workers with the same kind of forms. All present confusions would have been eliminated if former investigators had given a detailed description of this form

The oral opening of *O. viverrini* var. *Chiengmai* is nearly circular in transverse section, terminal and surrounded by the anterior sucker. The thickness of the oral sucker is about 0.13mm. Behind this there is a small pocket immediately followed by a circular pharynx 0.41 by 0.13 mm. The length varying from 0.13 to 0.15 mm, width constant. The length of the oesophagus in some cases is three times as much as the pharynx but this region is much at variance; from 0.24 to 0.42 mm with an average of 0.31 mm. At the base of the oesophagus there is an enlargement before the bifurcation takes place. The intestinal crura extends between the uterine coils and vitellaria nearly to the posterior end of the body. Usually both end at the same level. Sometimes the right or left extending further. The average distance from the posterior end to the end of the crura is 0.25 mm, varying from 0.11 to 0.4 mm.

The female reproductive organs are composed of multilobed ovary, the vitellaria on both sides of the margin with a transverse duct. The shell glands are composed of isolated cells opening into the ootype. The uterine coils dorso-ventrally and right to left in front of the ovary, and opens into the common genital pore immediately anterior to the acetabulum.

The vitellaria occupies a median position of the body from 0.03 to 0.13 mm from the body margin on both sides. The number of acini is regularly 8 on each side though it varies from 7 to 9 the extra one usually being on the right side. The right vitellaria

is larger than the left, it starts 0.38 mm from the posterior end of the acetabulum on the average. The closest it extends anteriorly to the posterior end of the acetabulum is 0.24mm, the farthest 0.9 mm. The left vitellaria 0.42 mm behind the acetabulum on the average varying from 0.3 to 0.84 mm. The range of extent of the vitellaria posteriorly varies little. It never extends behind the anterior testis more than 0.4 to 1mm on the right, 0.42 to 0.06 on the left. Taking the ovary for the comparison of vitellaria extent it is 0.06 to 0.42 mm below the anterior end of the ovary averaging 0.2 mm. Each acinus is connected by curved longitudinal ducts on each side. Later this duct unites with the transverse duct forming a broad V-shaped figure. The upper duct unites with a duct coming from the lower acini in front of the vitelline glands the united duct forms the transverse duct. The same kind of union takes place from the left vitellaria but not always in regular manner as sometimes the union from the upper and lower ducts takes place between or after they leave the glands.

The shell glands are somewhat diffused on both sides of the ovary and in front where the uterus begins. They are single isolated cells scattered in the parenchyma. They open into the ootype which is a little above the region where the vitelline duct opens into the uterus.

The ovary is multilobed, its margin smooth slightly toward the right(0.44 mm from the right side of the body 0.52 mm from the left). It occupies the anterior part of the posterior one-fourth of the body. The average width of the ovary is 0.46 and the length 0.22 mm varying from 0.34 to 0.57 mm by 0.19 to 0.29 mm

respectively. The number of distinct ovary lobes varies considerably from 3 to 9. Some of the lobes are much larger than others. There is always a regularity in having two branches, a right and a left one on each side of the receptaculum seminis resembling a saddle. The ovary is more antero-ventral to the receptaculum seminis and the lobes have three directions, right, left and median. In 80 per cent of the specimens examined the right and left lobes are equal. The oviduct opens into the uterus below the vitelline duct. Just above the common branch of Laurer's canal and the receptaculum seminis. The uterine coils occupy a median position in the middle 1/3 of the body. The uterus is filled with mature yellowish eggs. The lower end unites with the ootype and is mostly filled by spermatozoa. The genital pore is immediately anterior and median to the acetabulum, it is sometimes slightly toward right or left. The uterus opens in the common genital atrium by the side of the vas deferens.

The vas deferens coils from the right side and opens into the common atrium at the side of the female genital pore. The vasa efferentia coming from each testis unite between the uterine coils and form the vas deferens. The two testes are placed obliquely one behind the other. The anterior testis is four lobed, the posterior five lobed. The lobes are not always regular. From twenty specimens only one anterior testis had three lobes, in three the lobes were poorly developed the rest were well developed. There is greater irregularity of the posterior testis than the anterior one. In three specimens there was only four lobes, in another three, four others had five lobes but one or two of these were very poorly developed. The anterior testis

is more to the left side of the body, the posterior more median and somewhat inclined toward the right. Both testes are elevated from the level of the body and occupy most of the dorso-ventral space. The anterior testis is located in the 6th or 7th posterior tenth of the body at the posterior $\frac{2}{7}$ of the body. Its length varies from 0.19 to 0.48 mm. The average 0.34 mm. The width from 0.22 to 0.48 mm. The average 0.33 mm, the length and width of the posterior testis is 0.36 by 0.47mm, varying from 0.19 to 0.48 mm in length and 0.27 to 0.48 mm in width. There is a marked regularity in the position of the lobes, the anterior testis has four lobes and in 80 per cent of the cases these lobes are arranged right, left anterior and posterior. The posterior testis has five lobes and these are arranged anterior right, anterior left, right, left and posterior in 60 percent of the cases. In 20 per cent of the cases for anterior testis the tendency was anterior right, anterior left, posterior right and posterior left. In 30 per cent of posterior testis the tendency was anterior, anterior right, anterior left, posterior right, posterior left, in the rest of 10 per cent irregular.

The seminal receptacle is of medium size, pear or retort-shape and dorsal to the ovary; in all cases filled with spermatozoa. Laurer's canal starts from the common duct into which the receptaculum seminis opens and extends downward, curves backward, then to the right making a larger diameter to the tube, then it runs straight downward and opens on the dorsal surface of the body on the dorso-lateral side of the receptaculum seminis.

The excretory bladder is located in the posterior fourth of the body. It starts a little behind and lateral to the recept-

aculum seminis and latero-posterior to the ovary. It curves between the testes in an S-shape then enlarges greatly in diameter forming a bulb behind the posterior testis, it then narrows considerably and opens to the outside thru a narrow slit. The diameter at its anterior portion is about 0.48 mm, between the testes 0.23 mm, at the bulb region 0.4 mm at the narrow neck 0.15 mm, and at the pore 0.01 mm. These figures vary considerably.

The reproductive system - The genital pore opens immediately anterior to the acetabulum 0.008 to 0.018 mm. Mostly median though in some cases slightly to the right or left. The genital cloaca is about 0.07 mm long. The cloaca is flask shaped. The metratrum, the terminal portion of the uterus, has the same diameter as the cloaca at this region which is a continuation of the first. The ductus ejaculatorius narrows into a very narrow opening about one-half to one-third the size of the metratrum opening and at the side of it. The genital cloaca gradually narrows like the neck of a bottle. The genital pore opens at the level of the anterior of the acetabulum on the ventral side which is raised from the general level of the body. The wall of the cloaca is surrounded by heavy muscular annular muscles. Numerous prostate glands are located around the genital pore and open into it.

The vas deferens is filled with spermatozoa. It curves with the uterus side by side dorsally and opens in the genital cloaca 0.06 mm from the ventral surface or from the genital pore. The vas deferens starts from the anterior testis on the inner surface of the anterior lobe about 0.05 mm from the dorsal surface, it curves slightly above the testes and curves in at the shell gland passing between Laurer's canal and the intestinal crura and

under the transverse vitelline duct. The other vas efferens starts from the inner anterior right lobe of the posterior testis and joins the first at about 0.38 mm from the genital pore forming the vas deferens. The diameter of the vasa efferentia is about 0.01 mm the diameter of the vas deferens varies from 0.06 to 0.12 mm. The vasa efferentia unite between the uterine coils slightly (0.09 mm) dorsal to the median portion of the body. The vas deferens curves ventrally at the anterior margin of the acetabulum toward the dorsal side of the acetabulum. At the postero-dorsal region of the acetabulum it coils up till the posterior 1/3 of the acetabulum. Then it curves to the right on the same level and makes seven dorso-ventral loops before it reaches the vas efferens. The loops are situated on the right on the same level from the median longitudinal plane of the acetabulum. The loops are found in two regions one to the extreme right and the other more to the posterior. The extreme right loops are proximal to the acetabulum, they coil dorso-ventrally much more and are larger in diameter. The coils coming from the posterior of the acetabulum bend toward the right at about the same longitudinal plane with the lateral right margin of the acetabulum then coils dorsally. From there a short continuation to the posterior and to the right at that point it curves to the ventral surface then makes a short transverse landing. The last two coils are short and at the same level as the previous one. The posterior part of the vas deferens is straight and at the same level as the extreme left extension of the anterior coils.

The testes are obliquely placed on the posterior 2/7 of the body (average). The anterior testis more on the left and the

posterior more median or slightly toward the right. The lobes are short with round smooth margins. They originate from a common center. The length of the testes' lobes vary from 0.095 to 0.15 mm. The median portion of the testes filling nearly the dorso-ventral space at this region and are slightly closer to the dorsal than to the ventral surface.

The female reproductive system - The vitellaria occupies the lateral borders between the margins of the body and the intestinal caeca. The vitellaria never extend cephalad to the acetabulum nor caudad. Only in two cases do the vitellaria extend to the anterior margin of the anterior testis. The vitellaria do not quite occupy the middle 1/3 of the body margin. The mean relation in twenty specimens examined gave 0.42 mm anterior non-vitellaria portion, 0.34 mm median non vitellaria portion and 0.24 mm posterior non vitellaria portion. This ratio is in considerable variance in some individuals as the anterior portion extends somewhat in fixation.

The vitelline glands are oval or spherical, transversely placed in the parenchyma. A longitudinal duct from both vitellaria having ducts opening from each gland and later the two longitudinal ducts joined by a transverse vitelline duct which bends obliquely to the ovary and slightly toward the left of it enlarges forming the vitelline reservoir. In seventeen out of twenty specimens, the right vitellaria has 8 acini, 3 specimens show only 7, 16 have 8 acini on the left, two have 9 and another pair have 7 acini. At the beginning of the transverse duct, the longitudinal duct coming from the anterior and the one coming from the posterior form a long Y-shaped figure. On the right in

seven forms the union is among the glands, in the rest 0.038 to 0.057 mm. toward the ovary. On the left in 9 forms the union of the ducts is at the glands, the rest from 0.006 to 0.057 mm toward the ovary. In six forms the right acini were entirely distinct; on the left in one form seven acini were in combination, in two 5 acini and in three only two acini were in combination. In three specimens only three acini are in combination on the right. The space between the acini varies considerably but at a small margin from 0.05 to 0.12 mm the average being 0.09 mm. In six forms the number of acini posterior to the transverse duct on the right was two, the rest, one in the same region. On the left eight specimens have two acini the remainder having one. There is no break between anterior and posterior part.

The course of the longitudinal vitelline duct is at the inner margin of the glands median to dorso-ventral surface. Between the spaces of acini it curves toward the intestinal crura, the concave side being toward the glands. The width of the acini varies from 0.09 to 0.14 mm the length from 0.12 to 0.35 mm. In one case only an entire acinus was degenerated.

The transverse duct passes dorsally to intestinal crura. The transverse duct from both sides unites between the ovary and the seminal receptacle slightly toward the right. Branched efferent ducts from these groups of cells unite with the longitudinal ducts and these later with the transverse.

The vitellaria consists of numerous oblique oval, elliptical lobes with smooth rounded margins. In some species the lobes are very closely aggregated in others more or less distinct. Each lobe is surrounded by a thin membrane. The lobes are held

in the parenchyma. They contain numerous cells of shell forming material. These cells are mostly spherical and contain numerous yellow granules. There is a vesicular nucleus well stained with Erlich's acid hematoxylin. It has a distinct nucleolus and a number of chromatin granules.

The vitelline reservoir is on the left side of the ovary below the shell glands. The unpaired duct originates from the ventral anterior side of the vitelline reservoir. At the proximal end it proceeds anteriorly having a diameter of 0.018 mm at the distal end, where it opens into the common duct 0.04 mm in diameter. The unpaired duct opens behind the ootype, at this point it has a diameter of 0.06 mm. This measurement varies according to the amount of yolk found in the ducts. The thickness of the vitelline reservoir is 0.045 mm. It is formed by the expansion of the transverse duct in this region. The transverse duct continues dorsally while the unpaired duct below this proceeds at right angles to the transverse duct and anteriorly toward the ootype. This unpaired duct is in a plane 0.02 mm ventral to that of the transverse duct. The walls of the duct are homogeneous, no nucleus is seen. The thickness of the duct walls is 0.003 mm.

The ovary is multilobed and has a smooth margin. It occupies an anterior lateral and slightly antero-ventral position to the receptaculum seminis. The average length is 2.2 mm and the width 0.45 mm. The thickness varies according to the different lobes. The antero-ventral lobes are thickest 0.2 mm the others 0.12 mm. The oviduct proceeds anteriorly 0.01 mm to the right from the starting point where it joins the common duct behind the unpaired vitelline duct. The length of the oviduct is 0.36 mm.

It is 0.072 mm ventral to the receptaculum seminis. The duct coming from the receptaculum seminis and Laurer's canal curves making a small arch above the Receptaculum seminis, then forms a trough and begins to run straight anteriorly joining the oviduct. The ootype is at the posterior end of the uterus and is a slight enlargement in the diameter of the uterus. IN the ootype the shell glands pour their yolk and here fertilization of the egg takes place by the spermatozoa which pass from the receptaculum seminis uterinum into this region. The ootype is 0.12 mm from the ventral surface and 0.23 from the dorsal, its thickness 0.012 mm on the average. Its distance from the receptaculum seminis is 0.072 mm. The walls have thick annular muscles. They have nucleated cells. The lower part of the ootype is covered on the inside by cilia.

The eggs are oblique oval from a hundred measurements taken from twenty five specimens, four from each, sixty per cent gave 25 u for length and 15.6 u for width. In ninety-five per cent the maximum length was twenty-eight u and the remaining five per cent twenty-seven u, the rest 23.4 u the minimum length. The minimum width is 13 u and a width of 15.6 u. The width is more constant than the length. Laurer's canal from the upper dorsal side of the receptaculum seminis curves to the left side, takes a posteriorly course dorsally over the left lobe of the ovary., 0.18 mm below the receptaculum seminis. From there it passes from a dorsal to a ventral course by that making a curve anterior to the excretory bladder. Then it makes a slight curve and opens on the dorsal surface 0.054 mm from the excretory bladder. The diameter of the opening is 0.024 mm. The longest diameter 0.042

mm at the turning point from the ventral to dorsal surface. The distance from the origin to the dorsal opening is about 0.3 mm but the actual length is much larger as it curves much in its course. The walls of the canal is surrounded by circular muscles and cilia on the upper inner portion. At the opening to the outside there are glands opening into the canal. These appear to be degenerate prostate glands. This tends to show that Laurer's canal is a vestigial vagina.

Seminal receptacle is pear or retort shape. The length varies from 0.3 to 0.6 mm, the width from 0.18 to 0.26 mm, while the thickness varies from 0.2 to 0.27 mm. The receptaculum seminis lands from a median position toward the right side of the body in few specimens on the left and in one median. The anterior end is between the ovary lobes, the posterior part of it behind the ovary. The seminal receptacle is somewhat more toward the dorsal surface. The walls are non-nucleated. The sac-like cavity is filled with spermatozoa. The anterior end of the receptaculum seminis is tapering and very narrow about 0.018 mm. The Laurer's canal makes a junction at the anterior end. From this junction 0.06 mm on the left side is the vitelline reservoir. The oviduct opens just anterior to the Laurer's canal junction.

The uterus coils above anteriorly and to the right from the ootype and curves dorsally from this point to the ootype. There are no eggs in this portion which is however filled with spermatozoa. The uterus above the ootype is 0.024 mm in diameter. The uterine coils occupy nearly the middle 1/3 of the body. They are filled with yellowish eggs. The coils do not extend over the intestinal crura. The number of coils in different individuals

does not differ; the difference lies in the extent and the magnitude of the coils. In the long expanded individuals the coils are looser but in smaller and more contracted forms much more compact. From the metraterm the uterus prolongs dorsally and anteriorly to the left of the acetabulum at the lower margin of which it curves ventrally. It extends 0.036 mm posteriorly and coils dorsally and proceeds toward the right transversely making a dorso-ventral loop. From there it bends ventrally then dorsally passing transversely between the two crura which I call the first group. The margins of these loops are 0.12 mm from the left crus and 0.06 mm from the right respectively. The second group extends more toward the lateral crura, the left margin 0.18 mm, the right 0.036 mm. This group is formed by a transverse loop formed by the coiling of the uterus toward the left ventrally and toward the right dorsally. There are twenty-three such groups formed by the coiling of the uterus dorso-ventrally and transversely. Some of them are short and medium in size while others extend from one crus to the other.

The shell glands are single isolated cells scattered in the parenchyma anterior and antero-lateral to the ovary extending up to the uterus. They open into the ootype. Their secretions are known to be at present not shell material but yolk. The glands start nearly at the same plane as the ovary which is more ventral.

The excretory system - The excretory bladder occupies the posterior fourth of the body on an average varying from $1/3.3$ to $1/4.6$. The general shape is that of an S. The testes are located in the concave spaces. The bladder is well developed and occupies almost the dorso-ventral space except at the excretory

pore region, and the testes region $2/3$ to $4/5$ of the body thickness is occupied by the bladder. This must vary as in preserved specimens in most cases the excretory bladder is greatly contracted. Below the posterior testis the excretory bladder increases greatly in diameter becoming a circular bulb about 0.38 mm in diameter. There it narrows into a slit of 0.009 mm at the opening to the outside. The pore is posterior at the blunt tapering end of the body, the length of narrow slit is 0.05 to 0.07 mm. The shape of the excretory bladder in larval forms is straight but as the growing proceeds adjacent organs, the testes, expand and push the bladder aside to make more room for themselves causing it to take an S shape, the present form as was suggested by Ward. The ~~forming~~ of the anterior end in the genus *Opisthorchis* might be due to the same cause, that is to the growth of the ovary posteriorly.

There are three kinds of excretory ducts, two main ducts, one on each side of the body which carry waste material to the excretory bladder. One secondary duct unites with the main duct at the level behind the acetabulum the other at the front of the ovarial region. A branch comes from the posterior region of the body. A third kind of branches unite to the main and the secondary. These tubules terminate in flame cells. On account of the poorly preserved specimens I have not been able to follow every tubule. I have been able to see the flame cells in section. They measure 1 to 1.1 micra. The ducts and tubules are symmetrical on both sides of the body. The right main duct opens into the excretory bladder anterior to the posterior testis and the left 0.32 mm above the right branch. The main ducts have a very

wavy course between the intestinal crura and vitelline glands.

The alimentary system - The opening leading from the oral sucker into the pharynx measures 0.04 to 0.57 mm in diameter. The pharynx becomes somewhat larger in diameter toward the outer margin of the sucker. The oral opening is terminal, continued inside by the thinner cuticula. Behind the oral sucker there is a little pocket 0.048 to 0.076 mm in diameter lined by a thin lamella. The pocket is somewhat funnel shaped. Behind the pocket is more elliptical than the circular pharynx. The walls of the pharynx are much thinner dorso-ventrally than laterally so the pharynx has a verticle pit appearance when focused upon in the dorso-ventral position. The thickness of the pharynx is the same as the width. The inner lining of the pharynx consists of thin cuticula 0.0013 to 0.0026 mm in thickness. The opening of the oesophagus to the pharynx is much wider, about 0.047 mm varying from 0.036 to 0.092 mm. The oesophagus runs 0.03 mm ventrally to the middle line. It gradually increases in thickness and width 0.057 mm. The anterior end is circular gradually taking an elliptical form, the major axis being right to left. The average width of the bifurcation is 0.19 mm. These figures vary greatly according to the degree of contraction of the specimen at the time of fixation. The oesophagus has an inner homogeneous layer varying in thickness from 0.006 to 0.012 mm. The layer is surrounded on the outside by circular and longitudinal fibers. There is no columnar epithelial cells at the bifurcation and the oesophagus regions. The intestinal crura are lined with columnar epithelial cells which are embedded in narrow elongate cells with nuclei.

The crura prolong posteriorly at first more median from dorso-lateral surface and gradually bend to the dorsal 0.06 to 0.08 mm. They curve in their course right to left more than dorso-ventrally, but the later ones are greater in extent. It does not overtop the uterine coils but takes a course between the later and vitelling glands. It forms a trough in front of the testes and a crest above. The same happens at the receptaculum seminis due to the growth of these organs they push the caeca to one side. At the posterior end the crura bend slightly toward the median line of the body which is occupied by the excretory bladder. The crura in cross section are seen to be circular at the anterior region and somewhat irregular at the uterine and posterior regions. This can be expected as the uterus fills with eggs and consequently pushes the crura to one side, while the anterior region will be left normal. The crura end blindly nearly to the posterior end of the body. Generally both branches end at the same level 0.21 to 0.4 mm from the posterior end of the body 0.25 mm being the average. Sometimes the right or left extends slightly further. The length of the crura if straight would reach to the posterior end. The crura are always full of blood which shows that the parasite lives upon the blood of the host.

The cuticula - The body is covered by a non nucleated layer the thickness of which varies in different regions of the body. It being thicker at the oral end and around the ventral sucker. The pharynx, the oral sucker, the ventral acetabulum, the genital and the excretory pores are lined with a thin cuticular layer. A portion of the cuticula stains more intensely than the other, the deeper stained portion with Erlich's acid hematoxylin is the

outside layer and the lesser stained portion inside called by some the basement membrane. There are four different kinds of opinions as regards the origin of the cuticula. I would not attempt to bring all their arguments but will only mention them.

The coverings of the invertebrates is in general a true cuticula. Some authors think Trematodes and Cestodes have a cuticula secreted by the hypodermis which means morphologically that it is a true cuticula (Blochmann 1896). According to this theory epithelial cells are sunken into the parenchyma and connected to the cuticula by long projections.

A second view was that of Brandes (1892). He thinks the cuticula is secreted by the subcuticular single cells derived from the parenchyma and joined by ducts to the cuticula passing between the superficial fibers. Tennent (1906) adopted Brandes view.

A third theory is that epithelium (ectoderm) metamorphosed into cuticula. Wagner (1855). Some of the authors supporting this view are Monticelli, Goto, Nickerson and once by Braun. Schwarz working with cercariae and Zeller with Polystomum noticed that epithelial cells have their nuclei merely shrivelled.

Another theory originated by Leuckart (1886; 367) is that after the disappearance of the epithelium the parenchyma portions solidify in the peripheral regions by secretion of the cuticular substance from the whole body of the parenchyma. Looss (1893, 1894) Braun (1893: 818) Bratt (1898, 1899) and Young (1908) confirm this in their observations. Those who hold this view have various explanations to the possible function of the subcuticular cells. Pratt suggested they secrete some kind of protective substance against injurious products of the parasite. Looss and

Leuckart regard them as connective tissue structures which give rise to parenchyma cells. Looss compares it to the cambium of plants. By my study of this form I am convinced that the cuticula is formed as described by Young (1908:193) who worked with *Cystocercus pisiformis*. His view is identical to that of Leuckart. Quoting him "The cuticula of *Cysticercus pisiformis* is developed from a ground of simple parenchyma fibrillae by a deposition among them of a cement substance. There are no specialized fibrillae or cellular processes concerned in its development". I have seen those simple parenchyma fibrillae in the cuticula in different parts of the same section. I have seen it to be a continuation of the parenchyma fibrillae.

The parenchyma is formed of thin strands of connective tissue enclosing large vacuolated spaces. Between the fibrous net work there are small nuclei. All the body cavity is filled with these parenchyma cells. The parenchyma cells near the circular muscles are much smaller. It is the cortical layer of the ecto-parenchyma of Brandes (1892)

In the parenchyma there are numerous giant cells few in the pharynx and sucker muscles. These cells have many branches at each side two main branches coming from the central portion having the form of an epithelial cell nucleus. Each branch divides into main branches and extends to the longitudinal muscles, these cells are Nicoll's myoblast, ganglion cells of Looss. There are few of the same kind of cells as myoblast except the various branches radiate from the common center and each radiating branch divides into fine fibers. They also extend to the longitudinal muscles. These are called giant parenchyma cells.

In the form I have studied the subcuticular dells come next to the longitudinal muscles in the parenchyma. They are not arranged in a regular manner but are in a specific layer which even in the totomounts the subcuticular cell layer is well marked out. These cells have nuclei very much resembling the parenchyma cell nuclei but it seems to me this cell has a glandular nature rather than that of connective tissue structure giving use to parenchyma cells. Pratt (1909) thinks these cells have no ducts but are anastomosing branches which are interpreted as ducts. In my form I have seen very many spindle shaped cells their narrow ends toward the cuticula with a main branch which extends to the cuticula. Whether these branches are ducts or not I cannot definitely say, but I am inclined to think that they are, as these branches are continuations from the cell which in some cases clearly can be seen. But if these dells are glandular, what is their function? Do they secrete the cuticula as Blochman and others think? Pratt's arguments against that view are conclusive enough. Cuticula is found in every parasitic form but some ectoparasites have not the subcuticular glands. As there is no definite experimental evidence to prove the nature of these cells there is need of such work to prove their nature. They might secrete some kind of enzyme to assist the cuticula in counteracting the injurious substances existing in the host body.

Musculature - The muscles are fairly well developed. Especially the muscles of the oral sucker and acetabulum. The pharynx muscles are not as many as the sucker muscles. The suckers and the pharynx have three kinds of muscles; radial, circular and meridional or longitudinal, the radial muscles are the strongest.

In the body there is another additional kind of muscle the diagonal muscles passing from the right to the left or reverse. The muscles consist of bundles of very fine fibers in intimate contact with each other. In a sagittal section, the circular muscles are next to the basement membrane in a regular rectangular block. In cross sections these are hemispherical. Next to the circular muscles comes the longitudinal muscles. The diagonal muscles can be seen in the parenchyma and are much finer. The heaviest radial muscles pass dorso-ventrally. There can be seen in cross sections muscles extending from the acetabulum to the circular muscles called anchor muscles. The genital pore, the excretory pore and the oesophagus are surrounded by circular and longitudinal muscles.

The nervous system - The nervous system in this form is poorly developed. There is a central nervous system immediately posterior to the pharynx. It is composed of two ganglia on each side of the anterior end of the oesophagus united by a transverse commissure dorsally to the oesophagus. There are three branches running anteriorly, the inner dorsal, a median, and a ventral one to the oral sucker, an exterior and a lateral. Three nerves run backward from each ganglion, dorsal, ventral and lateral.

I have not been able to work out the complete nervous system but it seems it does not differ from other species of this genus.

DISCUSSION.

Diagnosis as given by Leiper (1915:573-575)

These worms resemble *O. felinus* but differ in the following points of specific importance. The skin is covered with minute acicular spinelets. The ovary is multilobate. The branches of the gut proceed almost to the extreme posterior end of the body. The ovary and the testes fill the last fourth of the body between the gut branches. The length of the oesophagus is from two to three times that of the pharynx, varying with the degree of contraction of the specimens. The ventral sucker is about equal in size to the oral, if anything occasionally slightly larger.

Host - Indian civit cat, and man.

"In Dr. Kerr's consignment of parasites obtained from prisoners in the Chiengmai jail was a tube containing three flukes regarding which the following notes were supplied" "*Opisthorchis felinus?* ova found in about fifteen percent of the faeces examined, The worms were twice obtained post mortem, in one case a single one from the intestine in another twelve worms from the gall bladder and large bile ducts. The ova have a small projection at the distal end, the cap. The worms also differ from the figures I have of *O. felinus* in having much larger part of the intestine unbranched, they also seem smaller, I was present at a post mortem by Dr. Mason where thousands of these worms were removed from the liver."

From the above quotation and the previous diagnosis of the form by Leiper who seems to have labeled the identification of the form as *O. viverrini*. My observations somewhat more in detail confirms Leiper's view. The form described in previous pages

corresponds to that of *O. viverrini*. A comparative study of *O. felinus* and *O. viverrini* will confirm this view.

O. felinus is much larger than *O. viverrini*; the former being from 8 to 13 mm the later 6mm. The form in question is 2.7 to 7.7 mm an average of 5.3 mm, corresponding to the later one as the 2.7 forms were very much contracted very many of the forms measured from 5 to 6 mm. The size of a form is a specific character. The view that the size of a parasite has variations with the size of the host is not accepted by prominent parasitologists. Looss, Braun and others. Looss (1907:42) against the view of Katsurado stated that every species of parasite has a size of its own. The occasional variation above normal is a case just as is found in the higher animals.

The next most striking difference between *O. felinus* and *O. viverrini* is in the size and shape of the ovary. In *O. felinus* the ovary is small simple or slightly lobed in *O. viverrini* it is larger and always lobed in the form under discussion. The seminal receptaculum in *O. felinus* is much larger compared to the ovary than in *O. viverrini*. The receptaculum seminis is less prominent than in *O. felinus* and relatively smaller than the ovary. Leiper in his paper reports the skin covered with minute acicular spinelets; although I have examined numerous specimens both toto mounts and sections, I have not been able to see any spines. I have noticed in the cuticula, or integument of some authors, deeply staining strands which might be looked upon as small spinelets but more careful study of a marginal sagittal section shows it to be of the same material as the walls of the parenchyma that is of connective tissue.

In *O. viverrini* Poirier the suckers are small and equal 0.28 mm in the var. Chiengmai nearly same as the above 0.19 by 0.224 mm the oral and 0.226 by 0.23 mm the ventral on the other hand *O. felineus* has 0.26 to 0.28 mm the oral sucker width and 0.228 mm the length, the acetabulum 0.247 to 0.228 mm long and 0.197 to 0.228 mm wide. The pharynx in *O. felineus* 0.204 to 0.169 by 0.160 mm to 0.170 mm in *O. viverrini* 0.14 by 0.13 mm varying from 0.13 to 0.18 mm in length and width 0.13 mm constant. Another marked difference between *O. felineus* and the two kinds of *O. viverrini* is the relative length of the oesophagus. In *O. felineus* it is very short nearly the size of the pharynx but in both varieties of *O. viverrini* the oesophagus is about three times as long as the pharynx.

The uterine coils in *felineus* are more compact and the coils do not correspond to that of *O. viverrini*. But one of the greatest evidences of these forms in question not being an *O. felineus* is in the difference of the eggs of the two forms. The two *O. viverrini* are exactly alike, though my average estimate for the *O. viverrini* are exactly alike, though my average estimate for the *O. viverrini* var. Chiengmai is a little greater than that given by Poirier. Poirier's form has a size 0.026 by 0.13 mm mine 0.026 by 0.0156 mm. Minimum size being 0.013 mm In *O. felineus* the egg length varies 0.026 to 0.030 mm and the width 0.011 to the 0.015 mm with an average of 0.030 by 0.011 mm That is in *O. felineus* the eggs are elongated and narrower, they have smooth outer operculum with the shell, but in both *O. viverrini* the shell at the operculum takes a flask neck appearance instead of a smooth round ending as in *O. felineus*.

In *O. felineus* the excretory system is Y-shaped in *O. viverrini* Poirier not described but from the picture it is most likely similar to the new variety, which is sigmoid. The primary ducts arising from different levels as represented in those figured.

Contrary to Looss's (1907:147) statement regarding the excretory bladder in the genus *Opisthorchis* being Y-shaped as one of the differentiating characters of *Clonorchis* from *Opisthorchis* the excretory bladder in this form is like that of *Clonorchis*.

Having in mind the different preserving methods and change in size of the forms accordingly in certain limits at certain parts especially I conclude that the difference between *O. felineus* and this form is distinct enough not to diagnose it as a new variety of *O. felineus* but one of *O. viverrini* Poirier. Some of the characters described above are constant enough to be regarded as specific peculiarities. Yet as Braun, Lühe, Mühlhng, and Looss when studying the forms of the genus *Opisthorchis* have spoken of the difficulty of distinguishing these species from each other, the same can be said for this new variety. In anatomical detail, the topography of *O. felineus* and this form have very much in common. In fact these forms have acicular spinelets as Leiper seems to have seen one cannot say it is not *O. viverrini* as Poirier in his description does not give any detail in regard to that. Yet there stands a fact which might keep the identity of these forms. Two forms might have identical features but by different species on biological ground, that is in the relation of the parasite to the host (Looss 1907:138). We know that *O. felineus* inhabits the cat, dog, gibbon, fox and man

but *O. viverrini* has not been reported before, as being a normal parasite of man. Poirier found the form from *Felix viverinius* in France if the form found from Siam is identical to that found by him we might expect human infection of the same kind in France which is not the case. The possible explanation in this case would have been simple if we could trace the history of the cat. But as no one has reported *O. viverrini* after that we might assume that it was an isolated case probably brought from other regions.

Supposing such an introduction from India suggests that there must be cases in which dogs and cats in India are infected which until now has not been reported as far as I am able to ascertain. There is no doubt from the circumstances in which these forms were secured that these are normal human parasites. But the similarity of the forms with *O. viverrini* Poirier and the possible case of being a normal parasite in cats too gives some certainty of its identity with *O. viverrini* Poirier. Thru the kindness of Dr. H. B. Ward I examined some of his collection of *O. felinus*. One form from the liver of man collected by Dr. Askanszy at Geneva, the other from St. Petersburg extracted by Kowalewsky from the cat. The two forms have some minor variations. The form living in man is much closer to the form in question than that from the cat, the sides run parallel and greatest width 1.1 mm but the form from the cat was much broader 2.9 mm. The oesophagus length 0.28 mm in the first one and only 0.228 mm in the latter. From this comparison I can see the explanation of the slight differences found between that of Poirier and mine. From Poirier's figure the form has not parallel sides but tapering at the anterior end gradually widening till the posterior end

which narrows and ends as in the variety described. In the figure given by Poirier the genital pore is placed much farther above the acetabulum than in forms I have examined. In his description Poirier states that the genital pore lies immediately anterior to the acetabulum so I regard the representation in the figure much farther than in fact it is. The posterior testis is described and figured as four lobed, but as Poirier does not mention how many forms he has examined I am inclined to think in the normal case it has five. From 50 individuals examined especially for this difference I found five with four lobes in many cases one of the lobes poorly developed. The position of the genital pore is constant and is specific but not the lobes of the testis. In the typical forms the anterior is four and the posterior five lobes but this varies within certain limits. In Leiper's figure the lobes in the posterior testis are much deeper than those I find in any one of my specimens otherwise the form is similar to mine except in none of the forms I have studied do I find any spines as Leiper stated to have found in his.

I add a few more records which might be of significance to explain the possible occurrence of *O. viverrini* in man.

In 1907 Verdun and Bruyant recorded the occurrence of *O. felinus* in a patient from Hanoï. The skin is stated to be smooth and the ovary round or arcuate. Maxwell sent from Formosa a macerated specimen from the stool of a cat to Leiper which had short spines on the cuticula. As the testes were digested it could not be decided whether it is an *Opiethorchis* or *Clonorchis*. Looss (1907:152) reports a form sent to him by Chun which was

poorly preserved and could not be identified. But the form was 4.5 mm in length 0.9 mm in breadth. He calls attention to a report from Ijima in 1886 which states that the same kind of forms having short spines in the skin were taken from the liver of a cat. This form was regarded as a different form of *Clonorchis* endemicus. Accordingly Looss said it might be another species. Leiper thinks it might be *O. viverrini* variety in question. One thing definite is this that *O. viverrini* Poirier is identical with the form in question and anatomically has very close resemblances to *O. felineus*. From a biological standpoint there is more definite proof this being a variety of *O. felineus* which is a normal parasite of man and also of the cat, that *O. viverrini* Poirier, which is reported definitely only from the cat. If it can be proven that *O. viverrini* var. *Chiengmai* is not parasitic in the cat then it will become a new species instead of a variety of *O. viverrini*. But from the data at hand I can with a certain degree of certainty say that this form is *Opisthorchis viverrini*.

But this only possible reasoning is less important if we bear in mind that Feline *Opisthorchis* may occasionally occur in man as stated by Looss (1907:152). Not only is this true in the case of *Opisthorchis* but in other forms as *Paragonimus westermani* and *kellicotti* are found both in the dog and in man, in Japan. Certain canine and feline parasites as *Coccidium bigeminum*, *Ascaris*, *Dipylidium* are parasites in man also. Therefore I diagnose this form as *O. viverrini* Poirier var. *Chiengmai*, a normal parasite of the Indian civet cat not recorded heretofore from man. In finishing I would express my thanks to Professor H. B. Ward for his valuable suggestions during this work.

SUMMARY

1. The history of the genus *Opisthorchis* is given.
2. Specific diagnosis of the forms belonging to the genera *Opisthorchis*, *Amphimerus* and *Paropisthorchis* is given with their generic diagnosis.
3. The relation of the species, the difficulty of their identification is discussed briefly.
4. The description of a human parasite belonging to the genus *Opisthorchis* is worked somewhat in detail and diagnosed as *Opisthorchis viverrini*., var. *Chiengmai*.

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1000. I have the honor to acknowledge the receipt of your letter of the 25th inst.

and in reply to inform you that the same has been forwarded to the proper authorities.

Very respectfully,
Your obedient servant,

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for the purchase of land

in the County of Albany

for the use of the State

in the name of the State

for the purchase of land

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VII. EXPLANATION OF PLATES.

Abbreviations

ac	acetabulum	os	oral sucker
am	anchor muscle	p	parenchyma
cgl	cephalic glands	pgl	prostate gland
cm	circular muscle	pd	pedicle
dan	antero-dorsal nerve	ph	pharynx
dm	diagonal muscle	pn	parenchyma nucleus
dvm	dorsoventral muscle	pph	prepharynx
eb	excretory bladder	rs	receptaculum seminis
eph	epithelial cell	rsu	receptaculum seminis uter- inum
es	oesophagus	sg	shell gland
gc	ganglion commissure	sgl	salivary gland
gp	genital pore	t.	anterior testis
it	intestinal crura	ta	posterior testis
lc.	Laurer's canal	ut	uterus
lco	Laurer's canal dorsal opening		
lm	longitudinal muscle	va	matraterm
mm	meridional muscle	vd	vasdegerens
my	myoblast	vdt	vitelline duct
od	oviduct	vr	vitelline reservoir
ov	ovary	vtd.	vitelline transverse duct
		vid.	vitelline unpaired duct.

PLATE I. - Fig. 1 *Opisthorchid sibiricum* (= *O. felineus*) magnified after Winogradoff from Braun. Fig. 2 Egg of *O. felineus*. Fig. 3. *O. felineus* (Riv.) after Braun magnified. Fig. 4 *O. geminus* x 10.5 Looss Plate 24 fig.2 From *Anas penelope*. Fig. 5. Egg of *O. geminus* x 631. Fig. 6 *O. simulans* x81 after Looss 1899. Fig. 7 Egg of *O. simulans* x 631 Fig. 8 *O. obsequens* x 24 after Nicoll. Fig. 9 *O. tenuicollis* x15 after Braun from Barker. (1911 pl 20 fig. 4.) Fig 10 Egg of *O. tenuicollis* Fig. 11. *O. longissimus* x10 after Stiles and Hassall from Barker. Fig 12. *O. viverrini* magnified after Leiper. Fig 13. *O. viverrini* x 17 after Poirier. Fig. 14. Egg of *O. viverrini* Fig. 15. *O. viverrini* central female organs. Fig. 16. *O. simulans* central female organs.

PLATE II. - Fig 17 *Opisthorchis geminus* x11 from *Anas boschas* Looss 1899. Fig. 18 *O. geminus* egg. Fig 19. *O. geminus* female central organs. Fig. 20 *Amphimerus pseudofelineus* x70 Ward (Reference handbook of medical Science Fig. 4772) Fig. 21. *A. pseudofelineus* Central female organs. Fig. 22. *A. pseudofelineus* egg. Fig. 23 *A. pseudofelineus* spines. Fig 24. *A. pseudofelineus* genital region. Fig 25. *A. ovalis* x16.5 After Barker . Fig. 26. *A. ovalis* spines. Fig. 27. *A. ovalis* egg. Fig. 28. *A. ovalis* genital region. Fig 29. *A. lancea* Diesing x17. ventral view after Weski. Fig. 30. *A. speciosus* x15 After Stiles and Hassall from Barber. Fig. 31. *A. speciosus* egg. Fig. 32. *A. interruptus* (from *Alcedo bicolor*) x20 After Braun. Fig. 33. *A. noverca* after Leuckart, from Braun. Fig. 34. *A. noverca* egg. Fig. 35 *Parapisthorchis caninus* x13 from Barker

Fig. 36. *P. caninus* spines.

PLATE III. Fig 37 - *Opisthorchis viverrini*, var. *Chiengmai* x37 ventral view. Fig 38. *O. viverrini* x30 dorsal view. Fig. 39. Female central organs x135 dorsal view. Fig. 40 Female central organs x 135 ventral view. Fig 41. Genital region x 100 dorsal view. Fig 42. eggs of *O. viverrini* x840. Fig. 43. An egg of *O. viverrini* x 800. Fig 44. A wax model x50 of the male reproductive system. Fig. 45. Wax model of genital atrium. Representing the opening of vas deferens and neotraterm into the common atrium. Fig. 46. A cross section x 340 representing the tall epithelial cells and surrounding narrow elongated nucleated cells.

PLATE IV. Fig. 47 A cross section of the acetabulum x340 the anchor, the radial meridional and circular muscles of the sucker represented. Fig. 48. A saggital section through the acetabulum representing the vas defenens and ductus ejaculatorus and muscles of the acetabulum. Fig. A saggital section thru the ovary and receptaculum seminis representing the dorsal opening of Laurer's canal the ootype in which open single isolated shell glands. Fig. 50. A cross section of acetabulum.

PLATE V. Fig. 51 A saggital section thru the oral sucker and the pharyns. x 100. Fig. 52. A cross section thru the pharyns x100. Fig 53. Cephalid glands x 840. Fig. 54. A cross section posterior to pharynx. Fig 55. Vitalline glands in the parenchyma x 840. Fig 56. A saggital section x 340 showing muscles. Fig. 57. The central nervous system x 100. Fig 58. The excretory system in part.

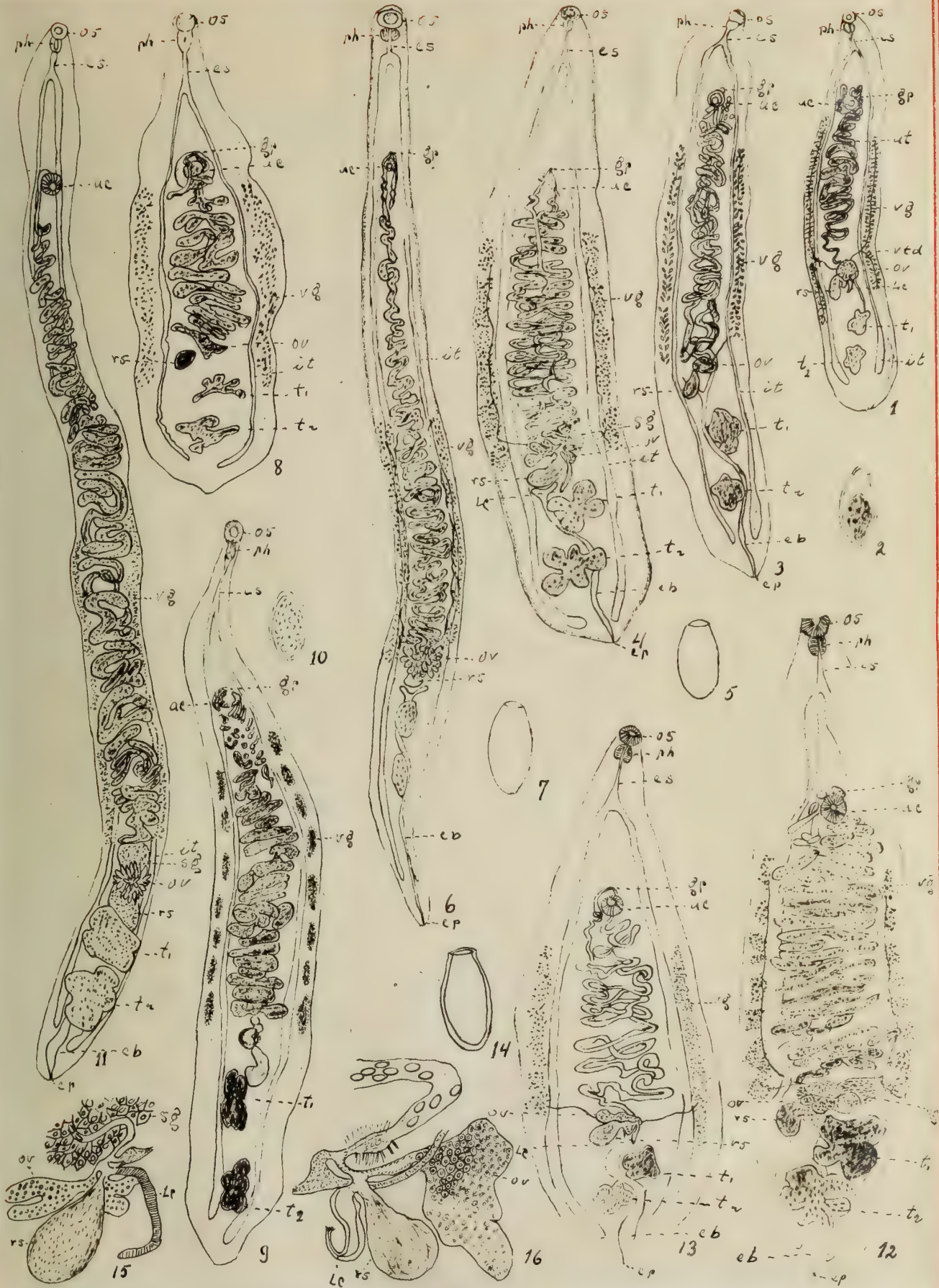


PLATE I



PLATE II.



PLATE III

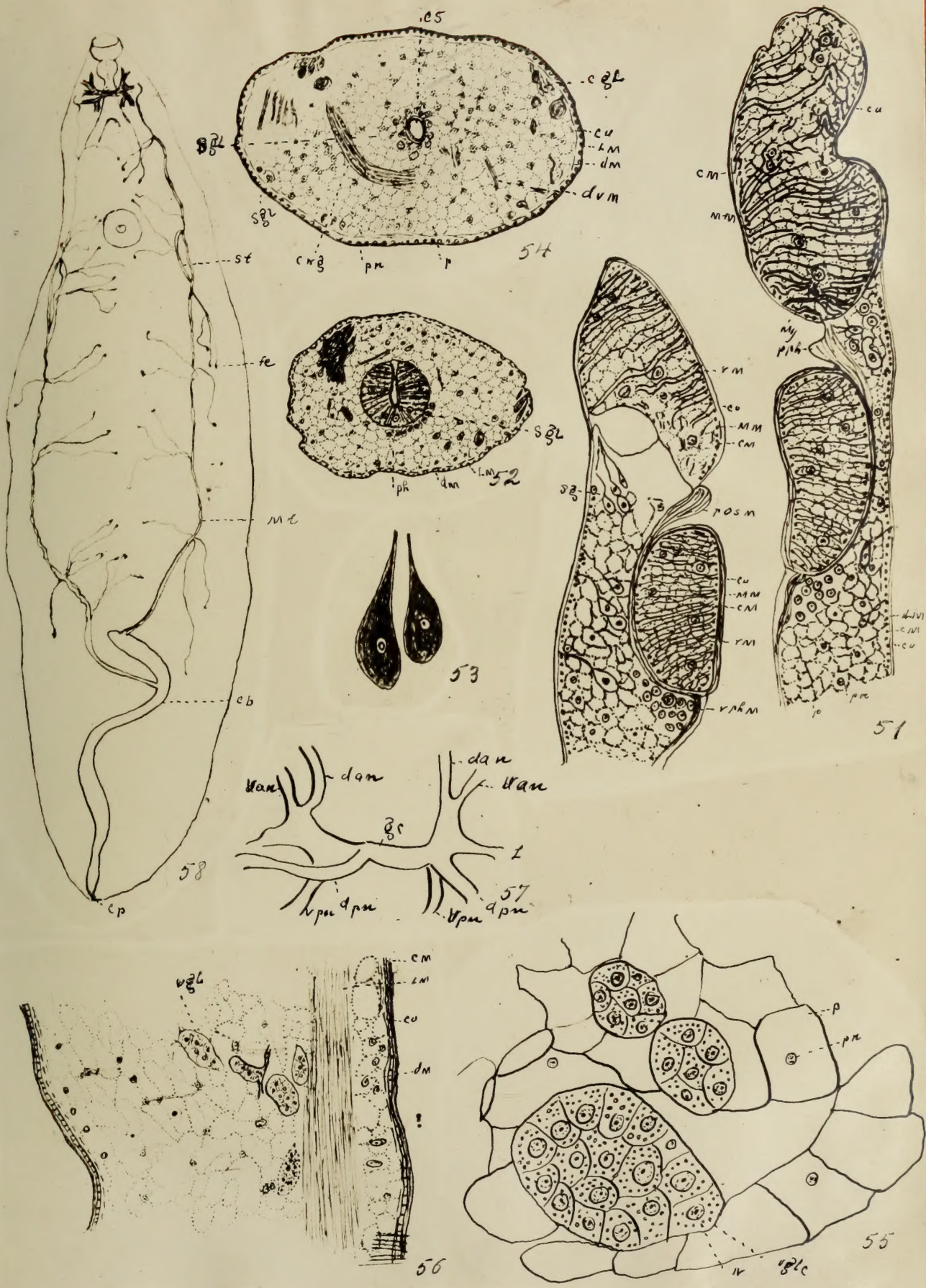


PLATE V

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